CALIFORNIA SEA GRANT

Biennial Report of Completed Projects 1988–90

A Publication of the California Sea Grant College

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The California Sea Grant College is a statewide, multiuniversity program of marine research, education, and extension activities, administered by the University of California. Sea Grant-sponsored research contributes to the growing body of knowledge about our coastal and ocean resources and, consequently, to the solution of many marine-related problems facing our society. Through its Marine Extension Program, Sea Grant transfers information and technology developed in research efforts to a wide community of interested parties and actual users of marine information and technology, not only in California but throughout the nation. Sea Grant also supports a broad range of educational programs so that our coastal and ocean resources can be understood and used judiciously by this and future generations.

Rosemary Amidei
Communications Coordinator

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Biennial Report of Completed Projects 1988-90

California Sea Grant College
University of California
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Introduction

This biennial report presents the results of research activities undertaken by the California Sea Grant College Progam during fiscal years 1988-89 and 1989-90. It is meant to be a technical record of our accomplishments for use by individuals in academia, government, and industry. This publication contains only reports of completed projects (as opposed to descriptions of work in progress). It thus forms an important historical record of program achievement.

For readers unfamiliar with our program, the California Sea Grant College Program is the largest of 29 Sea Grant programs underway in more than half the nation's states. Its purpose is clearly stated in the 1966 National Sea Grant College and Program Act responsible for its creation: "to increase the understanding, assessment, development, utilization, and conservation of the nation's ocean and coastal resources by providing assistance to promote a strong educational base, responsive research and training activities, and broad and prompt dissemination of knowledge and techniques."

California's Sea Grant College is administered by the University of California and is headquartered at Scripps Institution of Oceanography, part of the University of California, San Diego.

James J. Sullivan
Director

Coastal Resources

Prediction of Nearshore Sediment Transport Using a Model for Fluid-Sediment Coupling

University of California, San Diego
Scripps Institution of Oceanography
R/CZ-77
Project Initiated: October 1, 1986
Project Completed: March 31, 1990

Douglas L. Inman and Daniel C. Conley

Introduction

Inman et al. (1986) reported an idealized sequence in the development of the fluid-granular boundary layer under nearbreaking waves. This sequence could be divided into three quasi-distinct events—streaking, roiling, and oscillatory bursting—that appear to represent different flow stages in the growth and decay of the oscillatory boundary layer (Figure 1). Each of these stages appears to represent distinct transport regimes that are unique in their transport mechanics. Briefly, streaking begins with the onset of grain motion and is characterized by thin, rolling, grain-type motion in which only the top few layers of sediment are in motion and the thickness of the fluid-granular layer is on the order of a few grain diameters. Roiling is initiated by the abrupt transition from the thin, shooting flow of the streaking stage to a thicker (>1–2 cm) sediment laden sheet- or carpet-type flow. The fluid-granular layer now appears totally disorganized and fully mixed, although under the proper conditions, it can reorganize into a series of vortical roils of sediment that give the appearance of the wales in corduroy (Figure 2, cases A and B). These wales settle out into transition ripples when the deceleration subcycle ends with roiling. Oscillatory bursting is the ejection, from the crest of each roil, of a plume of sand and water into the fluid interior where it is transported as suspended load. In its most pronounced manifestation, this bursting can carry sand 10–15 cm above the bed (Figure 2, case C).

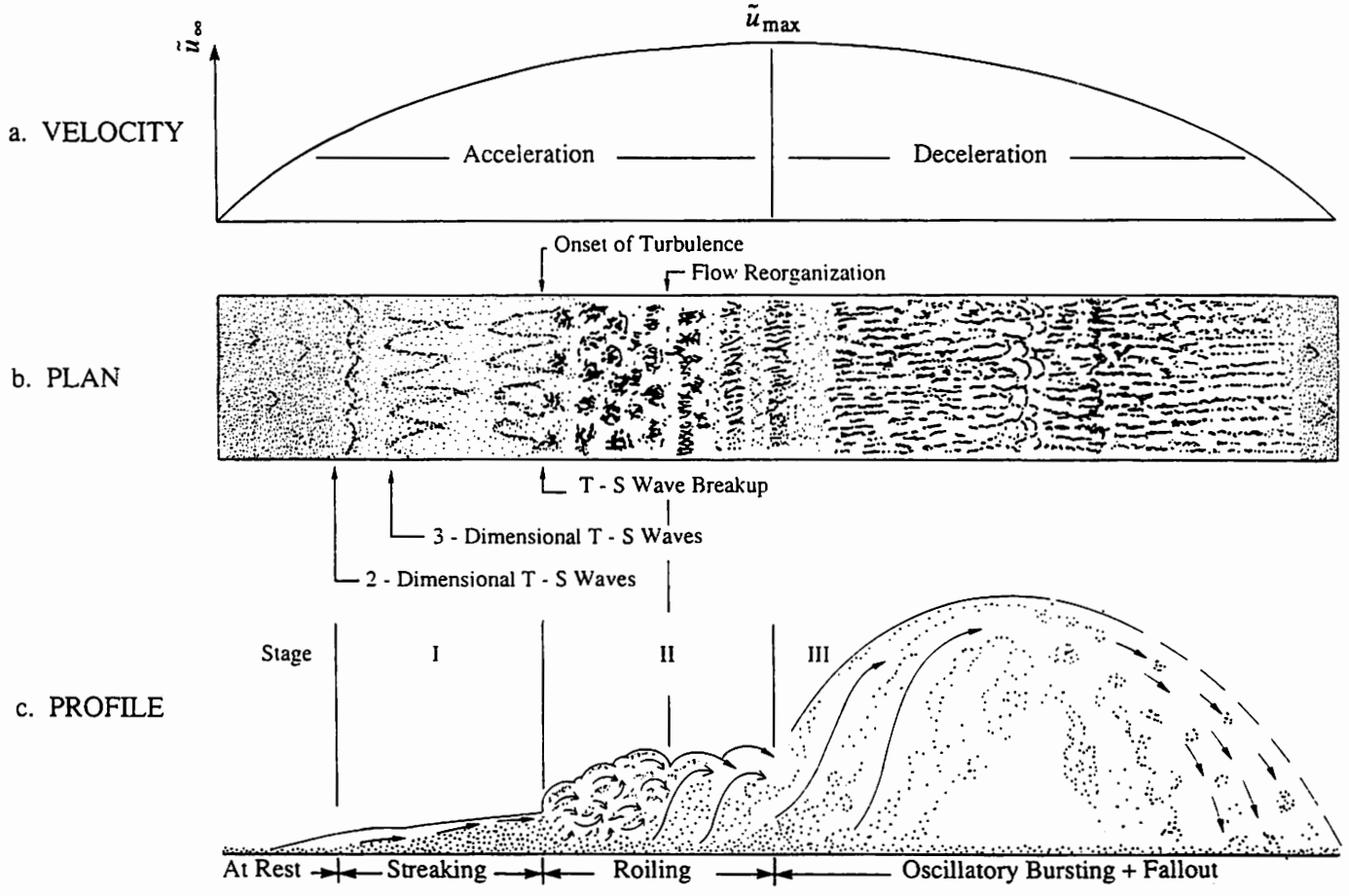

Figure 1. Schematic illustration of the sequences in the development of the fluid-granular boundary layer for the case of "fully developed" oscillatory bursting under the crest of a nearbraking wave (case C of Figure 2).

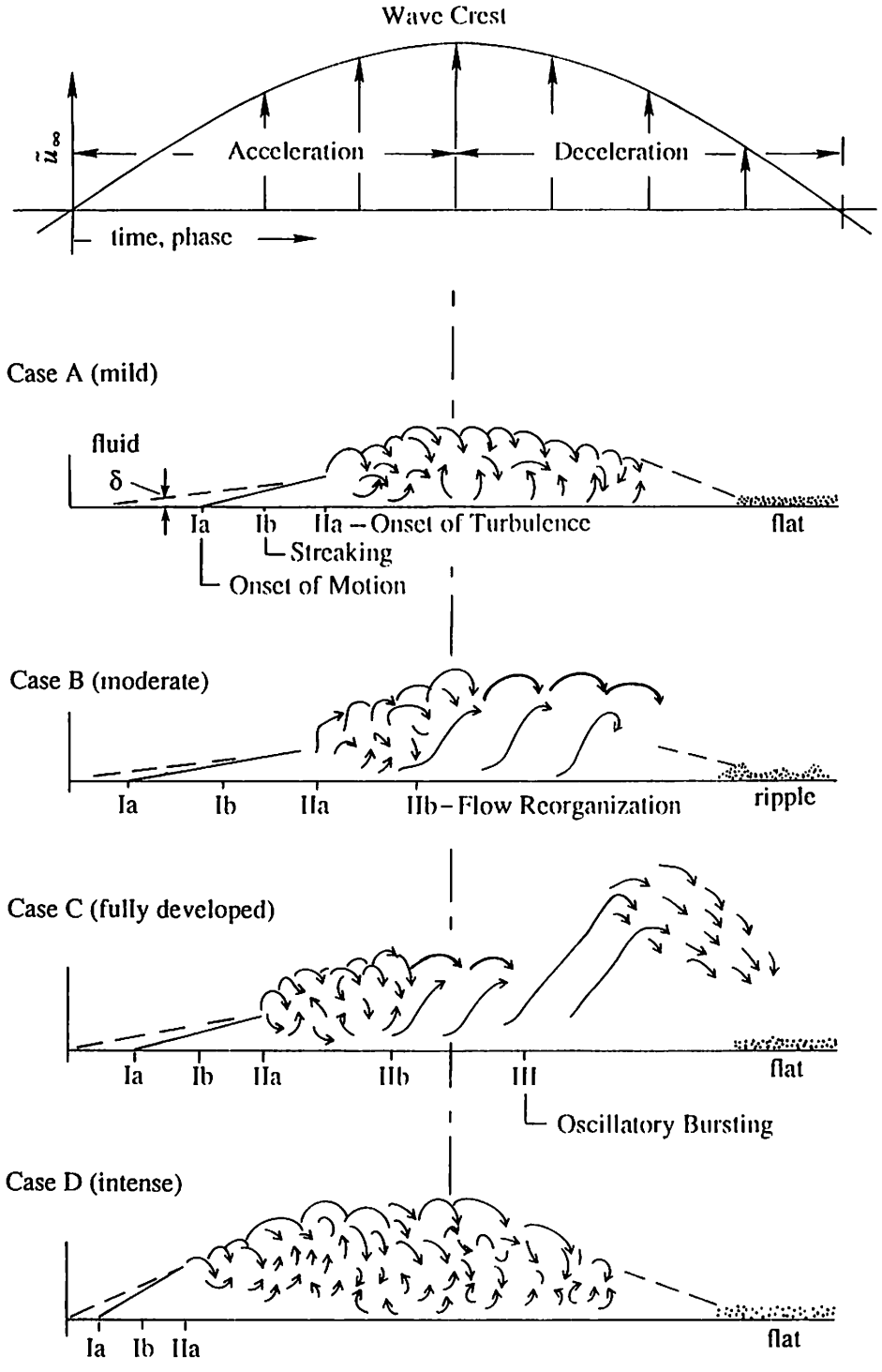

Figure 2. Schematic drawing of four identifiable sequences in the development of the fluid-granular boundary layer under nearbreaking waves, shown in the order of increasing intensity of motion at the interface. Details of case C are shown in Figure 1.

The Basic Model

The onset of these events in the developing boundary layer is associated with the characteristics of the potential, free-stream flow above the bed, which is generally laminar. In detail, the onset criteria for these flow events are undoubtedly functions of the frequency and nonlinearity of the waves and the type of bed material. However, for field experiments over beds of fine sand in water depths of about 2 m with waves of 8- to 10-sec periods, the onsets were associated with the magnitude of the free-stream velocity. This means that the regimes can be characterized by free-stream onset velocities designated as u_s, u_r, and u_b for streaking, roiling, and bursting, respectively.

A cross-shore sediment transport model has been developed. Its present form is based on the fact that for given wave condtions, the onset of each granular fluid regime is associated with a characteristic value of the free-stream velocity. By computing the immersed weight transport between the respective limits for each regime and then summing over these regimes, the total transport can be calculated. The transport rate can be expressed as,

$$i = (1/t_s)\int_{t_s} U_s m'_s dt + (1/t_r)\int_{t_r} U_r m'_r dt + (1/t_b)\int_{t_b} U_b m'_b dt ,$$

where U is the mean grain velocity; m' is the immersed weight of grains in motion over unit area of bed; t is the period of time transport occurs in each regime; and the subscripts s, r and b refer to streaking, roiling, and oscillatory bursting, respectively.

The model is evaluated by summing these time-dependent quantities for each regime. The mean grain velocity is computed from the free-stream velocity, u, as $U_i = c_i u$, where c_i is a regime-specific constant of proportionality. The values chose for these constants come from assuming logarithmic profiles of both velocity and sediment concentration in the boundary layer. Observations indicate that the rolling grain, streaking transport occurs in the bottom fifth of the boundary layer; roiling or sheet flow occurs throughout the boundary layer; and oscillatory bursting distributes sand to a height approximately five times the thickness of the roiled boundary layer. These observations, combined with the logarithmic boundary layer profile, result in $c_s = 0.162$, $c_r = 0.49$, and $c_b = 0.83$. Figure 3a shows this distribution of sediment velocity vs. fluid velocity.

The mass of grains in motion during roiling was assumed to be

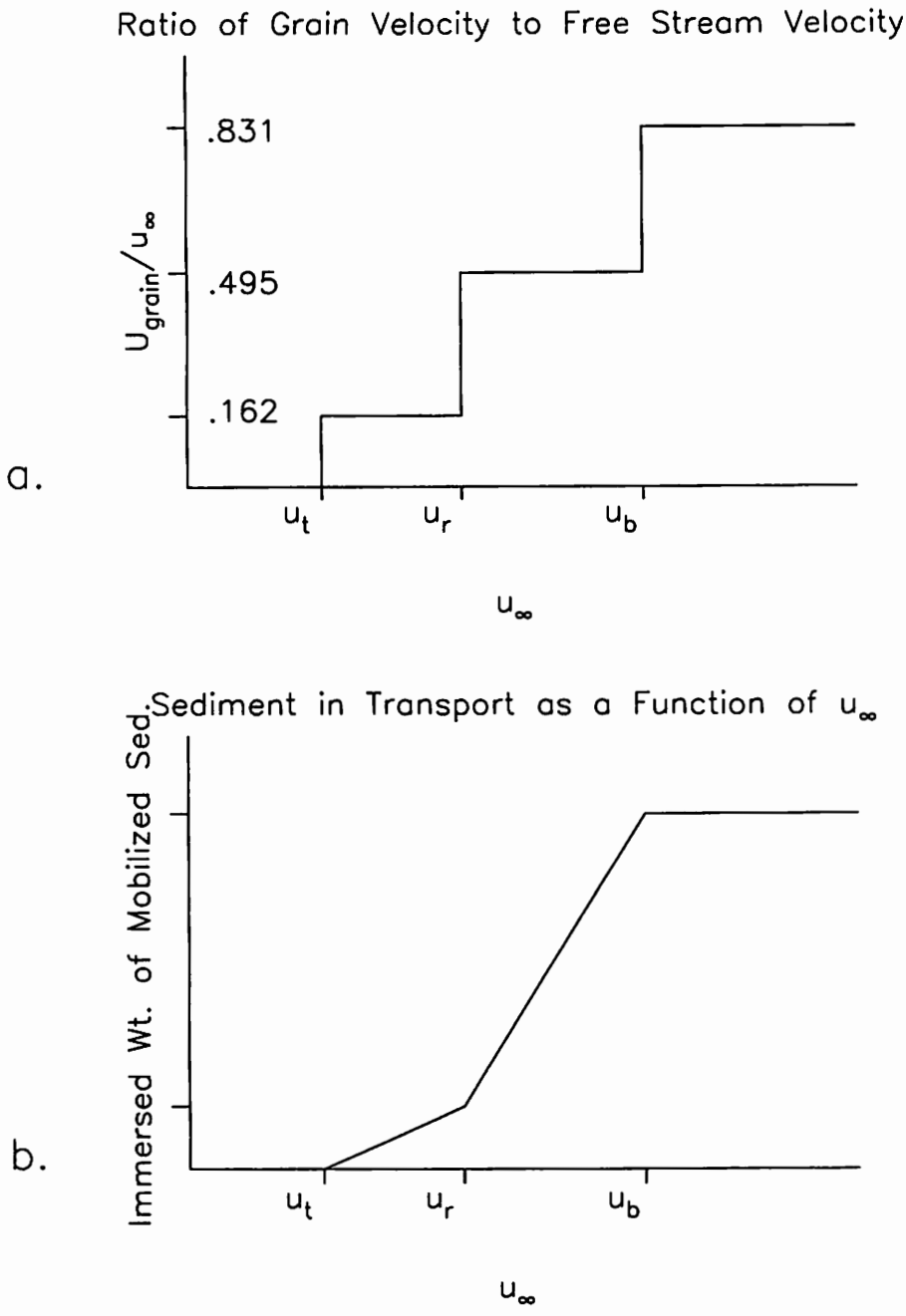

Figure 3. Distribution of grain velocity (a) and immersed weight of sediment in motion (b) as a function of free-stream velocity (u_∞) for model with initial assumptions.

proportional to the mass represented by the volume of sediment involved in ripple formation at the cessation of roiling. Thus, observations of the largest and smallest ripple volumes provide approximate upper and lower roiling mass limits, m_1 and m_2, respectively. Assuming a linear relationship between velocity and load, we can then calculate $m_r(u) = m_1 + a\,(|\,u\,| - u_r)$, where $a = (m_2 - m_1)/(u_b - u_r)$. Because the sediment involved in oscillatory bursting comes directly from the roiled layer, we assume m_b is equal to m_r at the critical velocity u_b (i.e., $m_b = m_2$). Finally, the mass of sediment involved in streaking is bound by the "at rest" lower limit of no sediment in motion and the lower roiled mass as an upper limit. An assumed linear relation results in $m_s = m_1(|\,u\,| - u_s)/(u_r - u_s)$. Figure 3b indicates how the immersed weight of grain varies as a function of fluid velocity. The immersed weight m′ of grains of mass m is calculated as $m' = (\rho_s - \rho)mg/\rho$ where ρ_s and ρ are the densities of grain and fluid, respectively, and g is gravity.

When a bed experiences oscillatory bursting, all the sediment involved in roiling is ejected from the bed and placed in the water column, where it moves with the free-stream velocity until it settles out at the cessation of motion. Therefore, t_b is defined as the period extending from the time $u = u_b$ to the following time that $u = 0$. With this definition, t_s and t_r are defined as the period during which $u_s \le u < u_r$, $u_r \le u < u_b$, except when that time falls within the definition for t_b.

Initial Assumptions

A computer program was developed for this model by using the velocity time series from a current meter as input. The program computes u_s from the wave period and sand diameter; then time steps through the time series, calculating the immersed weight transport at each step; and then sums and averages the transport for each regime. The program outputs the gross and net immersed weight transport for each regime as well as the total transport rate (Table 1). In addition, a time series of net transport in each regime is produced.

Initial verification of the model was done with all the current-meter time series available from the observations made during experiments on the development of a boundary layer. It was assumed that the beach profile was in equilibrium when the experiments were performed, and therefore the model should show zero cross-shore transport when tested on the data. When the model was run with these data, the resultant transport was an order of magnitude too large to be reasonable, so the mass of sediment in transport was reduced by a factor of 10. The predicted transport was now near enough to no transport to give preliminary validity to the model.

Quantitative testing of the model was to be performed by using the

Table 1. Output from Computer Program for Cross-Shore Sediment Transport Model

MODEL TRANSPORT VALUES FOR FILE twmmxa.dta

WAVE PERIOD(SEC = 9.1 VALUES COMPUTED OVER 11072.0 SECONDS

GRAIN DIAMETER(CM) = 0.0180

BEACH SLOPE = 1.15(DEG.), FALL VELOCITY = 1.90(CM/SEC)

Us/u = 0.162 Ur/u = 0.495 Ub/u = 0.831

LOWER ROILING RIPPLE VOLUME (DYNE/CM**2) = 13.220

UPPER ROILING RIPPLE VOLUME(DYNE/CM**2) = 74.030

STREAKING VELOCITY(CM/SEC) = 22.45

ROILING VELOCITY(CM/SEC = 45.00

OSC. BURSTING VELOCITY(CM/SEC) = 75.00

	Streaking	Roiling	Osc. bursting
TIME AVERAGED GROSS IMMERSED WT TRANSPORT (NT/SEC/CM)	0.104E-01	0.206E+00	0.160E+00
TIME AVERAGED NET IMMERSED WT TRANSPORT (NT/SEC/CM)	-0.240E-02	-0.137E+00	-0.885E-01

TOTAL TIME AVERAGED GROSS IMMERSED WT TRANSPORT (NT/SEC/M) = 0.3769E+00
TOTAL TIME AVERAGED NET IMMERSED WT TRANSPORT (NT/SEC/M) = -0.2275E+00

Table 2. Performance of Various Cross-Shore Transport Models

Model	Correlation Coefficient	90% Confidence Interval		Mean dynes/(cm-s)	Slope
		Upper	Lower		
Meyer Peter and Mueller (1984)	.820	0.93	0.56	0.6	0.34
Bagnold (1963)	.86	0.95	0.65	1.8	0.42
Yalin (1963)	.84	0.94	0.61	5.5	0.065
Bailard and Inman (1981)	.87	0.95	0.67	4.3	0.44
Hallermeier (1982)	.83	0.94	0.58	0.0	12.02
Madsen and Grant (1976)	.76	0.91	0.44	6.8	0.27
Present Model	.78	0.92	0.48	0.4	0.14

Note. Correlation coefficient represents how well the model performs. Slope is the slope of the regression line and represents a scaling parameter between model and measurements. Data are from the five sand-tracer experiments performed in 1984 by White (1987). Linear regression for the six energetics models is recalculated from White (1987) to apply to the same five runs used in this study. For the current model, the regression is performed on the final transport estimate as computed by using the revised assumptions and the original current-meter records.

extensive data set of sediment transport and current velocities reported by White (1987). After testing with this data began, it became clear that there was a problem with the data sets. Closer inspection of the field data showed that the data had been recorded with an inconsistent orientation for onshore flow. Therefore, it was necessary to inspect the analog records of the field data to determine the direction of onshore flow. This determination was based on the shape of the wave velocity profiles. Considerable time was used to identify and rectify this problem. Once the data were corrected, the model was run on the current-meter records from 5 different days, and the cross-shore transport was calculated. This transport was then compared with measured transport from sand-tracer studies performed on the same days (White, 1987). In this form, the model was sufficiently correlated with the data (correlation coefficient = .73) to provide encouragement to continue.

Revised Assumptions

While this model was being prepared, observations from additional experiments performed for this study and a related Office of Naval Research contract indicated a crest-trough asymmetry in the developmental sequence in the boundary layer. It was observed that true roiling with its characteristic three-dimensional surface structure appears to exist only under wave crests (onshore-directed flow) and that the fluid-granular boundary layer under the troughs was thinner and less dense under similar flow velocities. Additionally, it appeared that the offshore flow never showed oscillatory bursting. This suggested that the model should be modified to recognize the absolute direction of flow and differences in the development of a boundary layer associated with the crest and trough of the wave.

The model was adjusted in the following manner. Crest- and trough-flow regimes were treated equally up to and including the point of transition to roiling; after this point they were treated differently. In accordance with the new observations, the crest transport events were treated as before, but the trough was treated as a steadily developing, turbulent boundary layer. The mean grain velocity was taken as one-half (0.495) of the free-stream velocity, and the sediment mass increased linearly to some maximum value u_t, as shown in Figure 4. The mean grain velocity constant never increased beyond this value for trough flow, as observations indicated the absence of oscillatory

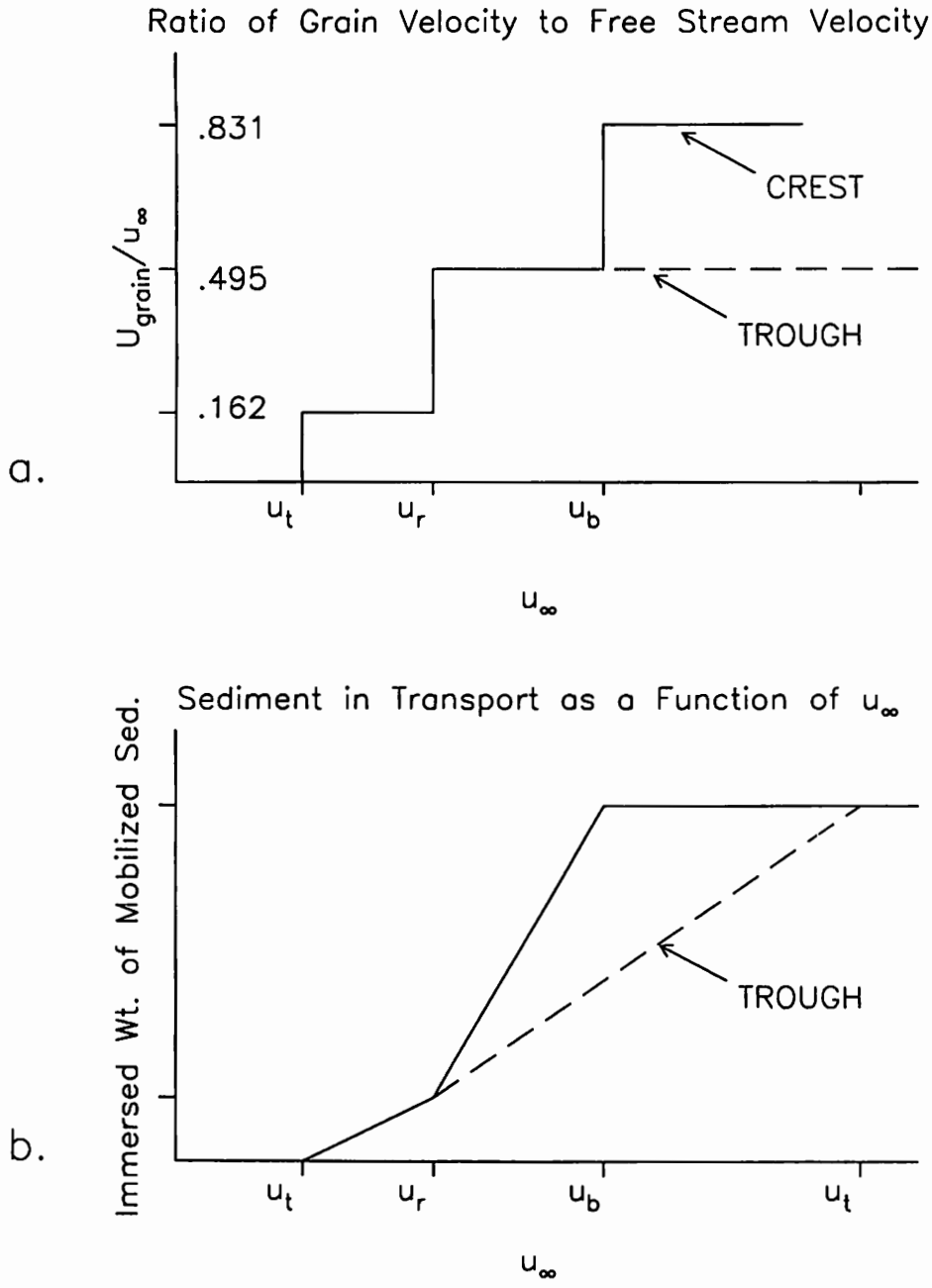

Figure 4. Distribution of grain velocity (a) and immersed weight of sediment in motion (b) as a function of free-stream velocity (u_∞) for model with revised assumptions.

bursting under these conditions.

This model was once again run on the five current-meter records and the predicted cross-shore transport was compared with the measured transport. The results with the model were not significantly different from the results of energetics-type cross-shore transport models computed by White (1987) (Table 2 and Figure 5). This is considered an encouraging result for a first attempt at such a phenomenological-type model.

Suggestions for Future Work

Greater understanding of the processes involved could lead to improvements in the performance of this model. Improvement is needed in determining coefficients c and m'; where c is the constant of proportionality relating the grain velocity to the free-stream velocity, and m' is the immersed weight of the mass of grains in motion. The mass of sediment in motion was deduced from still photographs and further adjusted in model trials by assuming that the beach was in equilibrium and, therefore, should have zero cross-shore transport. Accoustic Doppler-type bedload samplers now being developed should lead to improvement in knowledge of the mass in motion. This will free the model from the equilibrium assumption of no cross-shore transport, which may impose unrealistic constraints on the model.

The value of c depends on the thickness and type of flow in the boundary layer. Although boundary layer velocity profiles may not be exactly logarithmic, the deviation from logarithmic would not be sufficient to greatly influence this parameter. Also, the relative thickness of the layers in motion in each regime may be different from those used, but the values used are those that agree well with the observations.

In the second phase of the model, the control on sediment entrainment rate in the offshore direction is poor. It was decided that the model called for a slower rate of entrainment than onshore, so a velocity, u_t, greater than u_b, was chosen at which offshore flow would have entrained an amount of sediment equal to m_b. When this parameter (u_t) was varied, the correlation changed from a value of .77 to .79, as shown in Figure 6. There is at this time no control on this parameter, and a value of 120 cm/sec was chosen as the best for maintaining the entrainment rate associated with the streaking regime. It is also questionable whether a linear relation between velocity and mass of sediment in motion is appropriate. For example, if transport is proportional to stress, a squared dependency would be expected. Clearly, sediment entrainment is the weakest part of this development and would merit the most study in any attempt to improve such a model.

Inman et al., (1986) published one of the first descriptions of the development of the fluid-granular oscillatory boundary layer under field conditions. As such, it is clear that

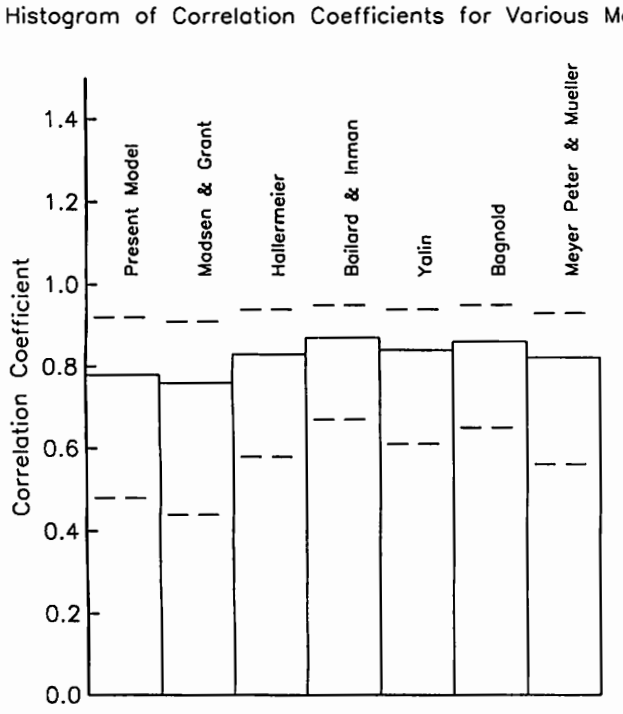

Figure 5. Histogram of model correlation with data for various cross-shore sediment transport models. Dashed lines represent 90% confidence intervals (refer to Table 2 for data sources).

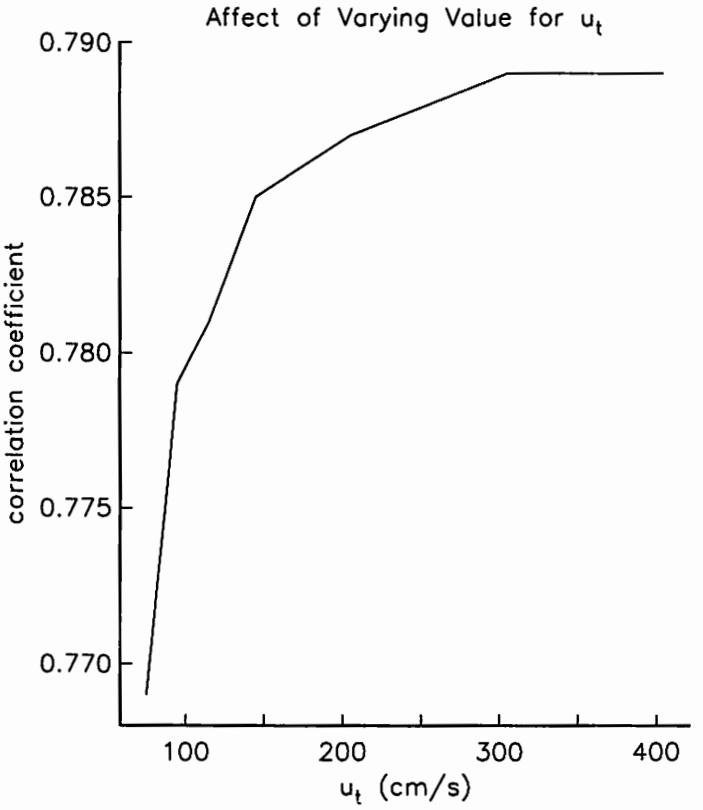

Figure 6. Model correlation with data as a function of u_t as defined in Figure 4b.

refinements of the description will be developed, and as they are, they can be used in the model to improve its performance. Addiitional observations that should lead to such refinements have been made during the course of the earlier mentioned experiments. These observations suggest that the transition from the thin shooting flow of the streaking regime to the thicker sheet or roiled flow depends on the transition to turbulence. If this is the case, the model could be modified to reflect the differences involved in turbulent vs. laminar sediment transport.

It is clear that improvements in models of sediment transport are going to come from modeling the true processes at work. This model was a first attempt at such a result. It was adequately successful at predicting the equilibrium beach profile and performed reasonably well (correlation coefficient = .78) for predicting measured sediment transport. It is thought that with improvements in the understanding of the processes involved in the development of a boundary layer, this type of model will eventually outperform the traditional energetics variety of models.

Cooperating Organizations
Office of Naval Research

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Maintenance of Entrance Channels of Coastal Lagoons and River Mouths

San Diego State University
R/CZ-79
Project Initiated: October 1, 1986
Project Completed: September 30, 1989

Howard Chang and Douglas Stow

The overall objectives of this project were to estimate sand-delivery rates for selected coastal streams in southern California and to study processes that influence the stability of small coastal inlets. Specific research goals for the project were as follows:

1. perform numerical model simulations of sand delivery by the Santa Clara and San Dieguito rivers and Buena Vista Creek and publish the results;
2. establish field-research and aerial-photograph acquisition programs at the entrance channels of the Tijuana River Estuary, Los Peñasquitos Lagoon, and San Dieguito River;
3. develop a better understanding of and attempt to quantify physical processes that occur at small coastal entrance channels;
4. modify the numerical model of stream flow to simulate tidal flows through small coastal entrance channels; and
5. incorporate meander migration into the simulation of sediment delivery by streams.

The State of California has a great interest in maintaining and enhancing the recreational and environmental values of its beaches, lagoons, and coastal streams. Also of great concern to resource managers in California is the preservation and, in many cases, restoration of coastal estuaries and their adjacent wetlands. Research undertaken in this project is helping to increase understanding of entrance-channel stability and will allow the testing of enhancement schemes for maintaining channel openings.

Much progress was made toward meeting the overall objectives of the project. Progress in meeting the specific goals includes the following:

1. completion of computer-model simulations estimating rates of sand delivery by the Santa Clara and San Dieguito rivers and the Buena Vista Creek to the sea, followed by publication of these results;
2. performance of topographic survey, hydraulic measurements, and aerial photography during numerous tidal flow events at the Tijuana River mouth and during a few events at the entrance channels of Los Peñasquitos Lagoon and the San Dieguito River;
3. analyses of wave, tidal, stream-flow, topographic, and aerial photographic data for the Tijuana River mouth to quantify the significant forces responsible for shaping the three-dimensional form of the entrance channel;
4. modification, testing, and use of the computer model of stream flow to simulate a tidal entrance channel by incorporating sediment-inflow from the sea and lagoon and by altering the effective tidal prism; and
5. development of a mathematical meander model to simulate meander migration and testing of the model by use of field data collected from an alluvial stream.

The application of mathematical modeling to the stream-delta-lagoon system is an important scientific advancement that also provides a useful tool for resource management (Stow and Chang, 1987a). By means of mathematical modeling, the convoluted relationships pertaining to the stream or tidal flow, sediment transport, and tidal variations are integrated to simulate the time-dependent processes for an actual stream-delta configuration. Such a model was developed, calibrated, and tested for conditions like those of small entrance channels in southern California.

The delivery and yield of coarse sediment (sand and gravel) in the Santa Clara River, San Dieguito River, and Buena Vista Creek were quantified through mathematical modeling of spatial and temporal variations of sediment characteristics for time-dependent, fluvial process-response (Stow and Chang, 1987b; Chang and Stow, 1988; Chang and Stow, 1989). The interactive effects on sediment yield due to sand and gravel mining, grade-control structures, and dams in the drainage basin were integrated into the mathematical modeling.

The sediment delivery to the coast is affected by the erosion and accretion in natural streams, which is characterized by a sinuous pattern. The migration of meanders and its effect on sediment delivery and budget are simulated by considering the secondary flow inherent in curved channels. This development supplements the model for sediment delivery: Now both the effects from meander channels and cross-section changes are incorporated into the model.

The methods developed as part of this project were applied for the Beach Erosion Authority for Control Operations and Nourishment (BEACON). The primary objective of the BEACON study is to make an estimate of the sediment delivery as affected by the existing dams and the ongoing mining of sand and gravel on beach-sand supply from the contributing streams in Santa Barbara and Ventura counties. Seven cities and counties are sponsoring members of BEACON.

Field surveys and aerial photography were acquired periodically at the entrance channels of the Tijuana River Estuary and the Los Peñasquitos and San Dieguito lagoons (Webb et al., 1989). These data provided one of the first comprehensive examinations of the morphodynamics of smaller tidal inlets from diurnal to seasonal time scales (Webb et al., 1990). It was clear that the stability of these inlets depend on large-magnitude, low-frequency wave and stream-flow events.

A model called INLET was developed for computer simulations

of physical processes in small tidal inlets. It was developed by modifying the FLUVIAL model for stream-flow processes to simulate the bidirectional tidal flow, changing sea and lagoon base levels, and sediment inflow from the sea and lagoon. The INLET model was calibrated and tested with data from field surveys for certain conditions. It was developed as a tool to assess the response of a small tidal inlet to various sediment-inflow rates representing inputs from storm waves.

The INLET model was used to study the morphological response of relatively small entrance channels to tidal-current, wave, and stream runoff processes, as well as the susceptibility of tidal inlets to closure. Results from validation tests for both neap and spring tide events at the inlet of the Tijuana River Estuary showed that the INLET model predicted terminal cross-sectional area ($R^2 = 0.91$) and change in cross-sectional area ($R^2 = 0.61$) with reasonable accuracy (Stow et al., submitted). In general, the shape of model-simulated cross sections were similar to surveyed cross sections. Storm runoff and cobble-size bed sediment were shown to have major influence on inlet morphology. The stability of the Tijuana inlet was assessed by simulating littoral sediment-inflow rates of 7.7, 76.7, and 383.5 m^3/day, which resulted in a stable open channel, closure after 120 hours, and closure after 55 hours, respectively.

The INLET model is also a valuable engineering tool for testing the effectiveness of artificial flushing schemes. The model was used to simulate the flushing capability of a tidal gate for maintaining an open inlet at the Tijuana River Estuary. Similar situations are ongoing.

Cooperating Organizations

Beach Erosion Authority for Control Operations (BEACON), a consortium of seven cities and counties in south-central California

California State Department of Parks and Recreation

Los Peñasquitos Lagoon Foundation

Philip Williams and Associates

U.S. Fish and Wildlife Service

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Stochastic Analysis of Estuarine Hydraulics

Humboldt State University
R/CZ-81
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Robert Willis, Brad A. Finney, and Mac McKee

Over the past 20 years, mathematical simulation models have been developed to describe the hydrodynamics and water quality of estuarine and tidal flow systems. The mathematical models are based on conservation of mass and momentum principles and describe the time and spatial variation in water levels, flow velocities, and water quality. Numerical techniques such as finite difference, finite element, and boundary integral or characteristic methods are commonly used to approximate the solution of the governing equations.

The validation and calibration of the mathematical models is predicated on the presumption that the hydraulic and geometric properties of the estuarine system (the boundary and initial conditions, parameters, and inputs) are known or can be inferred from observational data. In field applications of these models, parameter and input data are available only at a limited number of spatial locations and temporal intervals. Moreover, the information that is actually used in the modeling process is generally subject to measurement error. As a result, uncertainty in the data introduces a lack of reliability in the model's predictions (e.g., water depths and flushing times). Moreover, system inputs such as precipitation, wind velocity, and storm runoff are in themselves stochastic processes. A central question in estuarine modeling is how the uncertainty in the parameters and input data affects the response of the estuarine system.

One objective of this project was is to examine one aspect of uncertainty in estuarine systems: the effect of parameter uncertainty on the prediction of water levels and flow rates. A new approach involving quasi-linearization and perturbation analysis was used to approximate the solution of the random differential hydraulic equation. Explicit solutions were developed for the mean and variance of the water levels for any spatial location in the estuarine system. Monte Carlo methods also were used to analyze the effects of parameter uncertainty and to provide a cross check on the validity of the quasi-analytical solutions.

The overriding objective of this study was to determine the effects of parameter uncertainty on the prediction of water depths and flow rates in one-dimensional estuarine systems. In our summary (see Willis, Finney, McKee, and Miletelo, 1989 for complete summary and references), the solution of the stochastic differential hydraulic equations is approached by using a quasi-analytical procedure. Initially, the deterministic hydraulic equations are presented for a simple, one-dimensional estuarine system. Quasi-linearization is used to linearize these equations about trial solutions that represent the spatial variation in water depths and flow rates. Then, perturbation analysis is used to develop approximate solutions to the quasi-linear stochastic hydraulic equations. These solutions are approximated using finite difference methods. Explicit expressions also are presented for the mean and variance of water depths in the estuarine system.

Six conclusions can be derived from the results of this study.

1. In view of the general agreement between the Monte Carlo and quasi-analytical solutions, the quasi-analytical approach is a viable stochastic solution algorithm for the steady, gradually varied, open-channel flow equation. Provided that reasonable spatial increments are used, the quasi-analytical technique yields results that closely match the Monte Carlo simulation results.

2. The quasi-analytical method, predicated on quasi-linearization and operator analysis, requires significantly less computer time than does the Monte Carlo technique. For each Monte Carlo run, 1,000 simulations were performed to generate a reasonable sample with the proper distributional characteristics. The quasi-analytical model required convergence of the quasi-linearized hydraulic equations, and convergence was obtained in three iterations for each of the runs described in this investigation. Moreover, the computation of the moment equations required only one iteration before a solution was reached.

3. The quasi-analytical approach generates solutions for the water depths and flow rates at each point in the estuarine system as explicit functions of parameter or boundary condition uncertainty.

4. The spatial step size of the finite difference approximations affects the accuracy of the quasi-analytical solution as a result of the truncation error in the finite-difference approximation of the spatial derivatives. The truncation error is identical to that observed in a deterministic solution.

5. Results from the models indicate that, as the uncertainty in the channel roughness increases, the uncertainty in mean depth also increases. The predicted mean depth at a point in the channel decreases with increasing uncertainty in Manning's n.

6. The quasi-analytical method presented provides important planning information for the management of estuarine systems. The stochastic solutions provided by the quasi-analytical approach also can be used in the simulation and/or optimization of water levels and flow rates in open-channel flow or coastal-engineering problems. This information can be used to determine the risk or reliability of hydraulic control structures or alternative

discharge patterns.

Cooperating Organizations
Department of Civil Engineering,
University of California, Los Angeles

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Artificial Salt Marshes: How Well Do They Duplicate Natural Ecosystem Function?

San Diego State University, San Diego
R/CZ-82
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Joy B. Zedler and René Langis

Introduction

Where wetlands are filled or dredged for highway expansion, port development, marina construction, or other uses, resource protection agencies require mitigation through the restoration of degraded wetlands or construction of new wetland habitats. Emerging federal policies require that there be "no net overall loss of the nation's remaining wetlands base, as defined by acreage and function" (Conservation Foundation, 1988). Addition of the term "function" to mitigation policies means that replacement wetlands must demonstrate more than the short-term presence of transplanted vegetation. The functioning of replacement wetlands must equal that of the lost wetland site or suitable reference wetlands. To assist resource agencies in carrying out the no-net-loss policy, we explored functional equivalency of constructed and natural salt marshes within the Sweetwater Marsh National Wildlife Refuge and developed a Manual for Assessing Coastal Wetlands in Southern California. The manual was reviewed by agency representatives and was published in 1991. It discusses the need for wetland restoration projects to reach functional equivalency; summarizes the Sweetwater Marsh study; recommends standard methods for the analysis of soils, vegetation, and animals; and provides reference data from several of the region's natural wetland remnants. In this report, we evaluate the functional equivalency of the Caltrans Connector Marsh, which was to provide habitat for the endangered light-footed clapper rail. Cordgrass (*Spartina foliosa*), the cover preferred by this resident bird, was planted throughout most of the restoration site, and our comparisons focused on this "lower marsh" habitat. We report on three major functions: the dynamics of nitrogen and sulfides in the marsh soils, the growth and persistence of vegetation, and the support of food chains.

Study Sites

The study site (Figure 1) is located within the Sweetwater Marsh National Wildlife Refuge (32°38'N, 117° 6'W). The refuge is the largest remaining wetland complex in San Diego Bay; it consists of 128 hactares, most of which is salt marsh. An aerial photograph from about 1928 indicates that the entire site was part of the alluvial outwash from Sweetwater River. Dredging

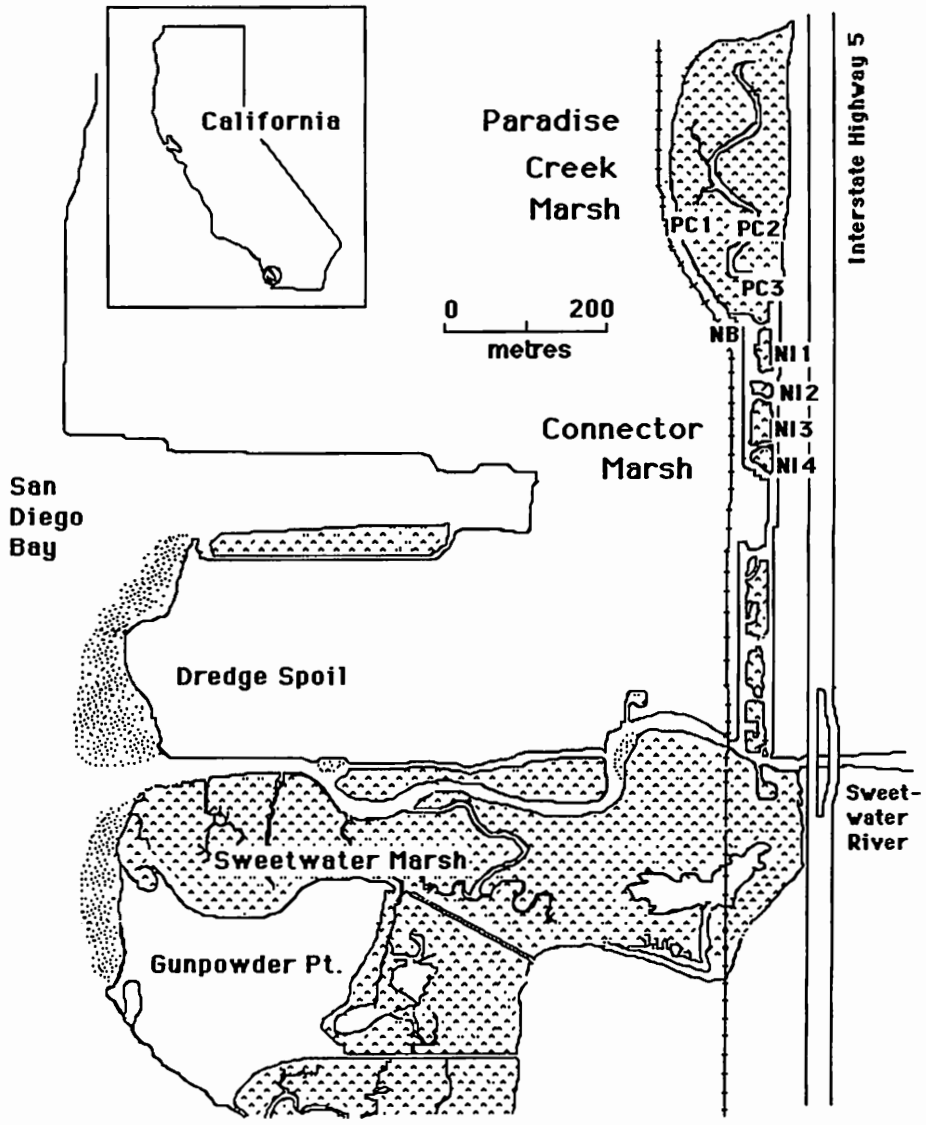

Figure 1. Map of the constructed Connector Marsh and the natural Paradise Creek Marsh. Location of sampling sites: PCI, PC2, and PC3 (Paradise Creek); NI1, NI2, NI3, and NI4 (Connector Marsh); NB (North Bank).

and filling along the bayfront, and use of the wetland as a dump site, raised the topography. Removal of fill was accomplished by the California Department of Transportation as mitigation for widening of Interstate Highway 5, immediately east of the study site.

The constructed salt marsh, known as the Connector Marsh, covers 4.9 hectares of formerly disturbed high marsh, salt flats, and upland terrain. In the fall of 1984, the site was graded to create eight lower-marsh islands and surrounding creek banks, which were planted with *S. foliosa* from January to March, 1985. The constructed site was fertilized on four occasions in 1985–1986 with urea at 25 g N/m^2 (H. Hunt, California Deptartment of Transportation, personal communication).

We restricted the choice of a reference wetland to a natural marsh immediately north of the constructed wetland. There were no tidal barriers between the northern portion of the Connector Marsh and Paradise Creek Marsh: a tide gate separated the northern part of the Connector Marsh from the southern part, and the Sweetwater River separated the Connector Marsh from other natural wetland remnants. Nutrient functions were assessed over 17 months, from December 1987 to May 1989, when the constructed marsh was 3–4 years old. Salinities of interstitial waters were 27–38 ppt in the constructed marsh and 23–36 ppt in the natural marsh.

Methods and Results

Dynamics of Nitrogen and Sulfides in Marsh Soils. We evaluated nitrogen dynamics by comparing nitrogen pools and nitrogen-fixation rates of constructed and natural salt marsh areas dominated by *S. foliosa*. Studies were restricted to a single intertidal elevation (0.43 m NGVD) and to adjacent wetlands, in order to reduce hydrological differences between the comparison sites. Nitrogen pools were examined in the sediment (total nitrogen, extractable ammonium and nitrate-nitrite, and organic carbon) and in pore water (ammonium and nitrate-nitrite). Soil samples were incubated *in situ* to evaluate the capacity of each marsh to transform organic nitrogen into available nitrogen forms. Nitrogen fixation was assessed in the surface soil (to 1 cm) and in the rhizosphere (to 10 cm). Experimental amendments (glucose and detritus) helped determine that microbial nitrogen-fixers were limited by carbon supplies. Additional soil measurements included texture, levels of organic carbon, redox potentials, and concentrations of sulfide.

Soil texture was coarser in the constructed marsh (loam to sandy loam vs. clay loam in Paradise Creek Marsh; Swift, 1988). Redox potentials were higher in Connector Marsh than in Paradise Creek Marsh on all dates except May 1989, which is also when differences between mean ammonium concentrations were lowest (Table 1). Samples for sulfide levels in sediments in the constructed and adjacent natural marshes were obtained near the seaward edge of cordgrass growth. The presence of only trace amounts of free sulfide in Connector Marsh (Cantilli, 1989) suggests that its biogeochemical functioning is not equivalent to that of a natural salt marsh. Concentrations of free sulfide (H_2S, HS) in the natural marsh were significantly greater at 5-, 15-, and 25-cm depths, with levels of nearly 3 mM at 25 cm. However, data on cordgrass aerial biomass of these San Diego Bay marshes and Tijuana Estuary suggest that free sulfide is not directly phytotoxic. Low concentrations of sulfide in the man-made marsh may affect energy flow and carbon export, as well as retention of heavy metals and sulfur.

Ammonium levels in the pore water (Table 1) were significantly higher in the natural than in the constructed marsh and an order of magnitude higher on every sampling date except May 1989. The lower value obtained in May could be a result of increased uptake of ammonium during rapid plant growth in spring (plants grow at an exponential rate during that period at Tijuana Estuary; Winfield, 1980).

Levels of nitrate-nitrite in pore water were generally low (Table 1), which was to be expected for Paradise Creek Marsh because of the highly reduced condition of its sediments. The low values obtained are indicative of that marsh's overall lower nitrogen status. Differences in nitrate-nitrite concentrations between marshes were significant on two of the four sampling sessions (Table 1).

Levels of extractable ammonium were significantly higher in samples from Paradise Creek Marsh than in samples from Connector Marsh for soil samples collected in May 1988 and 1989 (Table 2). This difference was of the same magnitude (3x–4x difference) as for the samples of pore water collected in May 1989, when the demand for nitrogen by the rapidly growing *S. foliosa* was high. No measurable levels of nitrate-nitrite were detected in May 1988, which indicates active denitrification or nitrate reduction during that period. Differences in extractable nitrate-nitrite were not significant in May 1989. Extractable nitrate-nitrite was, as in the case of pore water, much

Table 1. Concentrations of Inorganic Nitrogen in Pore Water of San Diego Bay Marshes

	Ammonium		Nitrate-Nitrite	
Date	CM	PC	CM	PC
September 1988	0.28 ± 0.07	2.36 ± 1.01*	0.06 ± 0.01	0.08 ± 0.01
November 1988	0.15 ± 0.03	1.74 ± 0.51*	0.08 ± 0.04	0.04 ± 0.51
February 1989	0.11 ± 0.01	0.95 ± 0.37*	0.12 ± 0.05	0.18 ± 0.06*
May 1989	0.15 ± 0.03	0.42 ± 0.28*	0.07 ± 0.01	0.10 ± 0.02*

Data are means (± SE) for 20 replicates at the constructed Connector Marsh (CM) and 12 replicates at the natural Paradise Creek Marsh (PC). Values are milligrams of nitrogen per liter. Significant levels (P) determined by Student's t test; * = significant differences for CM and PC ($P < 0.05$).

Table 2. Nutrient Pools and Organic Content in Sediment Samples of San Diego Bay Salt Marshes

a. Extractable inorganic nitrogen (mg N/kg dry weight of soil x 10^{-3})

Year	Ammonium		Nitrate-Nitrite	
	CM	PC	CM	PC
1988	1.26 ± 0.27	4.12 ± 1.64	not detected	not detected
1989	1.00 ± 0.16	4.08 ± 0.33	0.26 ± 0.03	0.21 ± 0.02

b. Total Kjeldahl Nitrogen (TKN, mg N/g dry weight) and percent organic carbon

	TKN		Percent Organic Carbon	
Year	CM	PC	CM	PC
1988	0.87 ± 0.04	2.01 ± 0.09	1.11 ± 0.10	2.38 ± 0.13
1989	0.94 ± 0.06	1.74 ± 0.13	1.08 ± 0.07	2.11 ± 0.15

Data are means (± SE) for 20 replicates at the constructed Connector Marsh (CM) and 15 replicates at the natural Paradise Creek Marsh (PC). Sampling was done in May 1988 and April 1989.

lower than extractable ammonium. Levels of total nitrogen and organic carbon were higher in the natural marsh than in the constructed marsh (Table 2).

Total nitrogen contents of *S. foliosa* above ground were significantly lower ($P < 0.005$) in the constructed marsh than in the natural marsh, but values for total foliar phosphorus were not. Taking into account the difference in biomass between marshes (453 g dry weight/m^2 in Paradise Creek Marsh and 192 g dry weight/m^2 in Connector Marsh), we calculated standing crops of nutrients for each marsh. Aboveground crops of nitrogen and phosphorus were approximately two to three times higher in the natural marsh (5.93 g N/m^2 and 0.67 g P/m^2) than in the constructed marsh (2.11 g N/m^2 and 0.30 g P/m^2). Average N:P ratios (molar basis) were higher in the natural marsh (19.7) than in the constructed marsh (15.6), which suggests greater nitrogen limitation in the constructed marsh.

The *in situ* experiment showed that inorganic nitrogen compounds, mostly extractable ammonium, increased more than 10 times during incubation in both marshes, which indicates that mineralization was as active in the constructed wetland as it was in the natural one.

Ammonification accounted for almost all of the nitrogen mineralization, with net nitrification being much less important. No significant decreases in the percentage of organic carbon and total Kjeldahl nitrogen occurred after 21 days of incubation. This indicates that inorganic nitrogen losses by ammonia volatization and denitrification (possible because the plastic bags we used were gas permeable) were not important. Overall, these results indicate that the supply of inorganic nitrogen to the system could not be limited by the mineralization process per se. Therefore, the differences in the inorganic nitrogen pools (in pore water and in sediments) must be explained by the difference in mineralizable organic matter, which most likely represents a small portion of total nitrogen or organic matter.

Nitrogen-fixation rates at the sediment surface were significantly higher in Paradise Creek Marsh than in the Connector Marsh sites in five out of six series of assays. Results obtained from the 10-cm soil cores, which included nitrogenase activity in the rhizosphere, showed a different pattern. Although nitrogen-fixation rates were significantly higher in the natural marsh in December 1987, differences were not significant on the three following assay dates, and rates were significantly higher in the constructed marsh in September 1988. The discrepancy in results for surface and rhizosphere assays can be explained by the relatively high levels of soil ammonium in the natural marsh. The presence of available nitrogen inhibits nitrogen fixation (Teal et al., 1979). The high levels of nitrate-nitrite in pore water (Table 1) and extractable ammonium in sediment samples from Paradise Creek Marsh (Table 2) support this explanation. On the other hand, concentrations of ammonium are typically much lower near the aerobic surface sediments, where oxidation and nitrification are active (Mitsch and Gosselink, 1986).

Low annual rates of heterotrophic nitrogen fixation (assuming a 365 days/yr and 24 hr/day activity period) in both the constructed and natural marshes (4.28×10^{-5} to 5.36×10^{-3} mol $C_2H_2 \cdot m^{-2} \cdot yr^{-1}$) indicated that nitrogen fixation contributes little to their nitrogen budget. These rates were much lower than those reported for Atlantic and Gulf coast salt marshes (5.14×10^{-2} to 10.9 mol $C_2H_2 \cdot m^{-2} \cdot yr^{-1}$; Howarth et al., 1988).

Soil organic carbon was consistently higher in the natural marsh. In addition, the percentage of organic carbon was highly correlated with total sediment nitrogen ($r = 0.95$), which indicates that it is an important potential source of available nitrogen. Organic carbon content was also significantly correlated with rates of nitrogen fixation ($r = 0.74$); this was expected, given that organic carbon is an essential energy source for nitrogen fixation.

Experimental addition of organic matter in the form of *Spartina* detritus (roots and rhizomes) had a significant effect on the rates of nitrogen fixation (Figure 2), showing the importance and potential contribution of organic matter

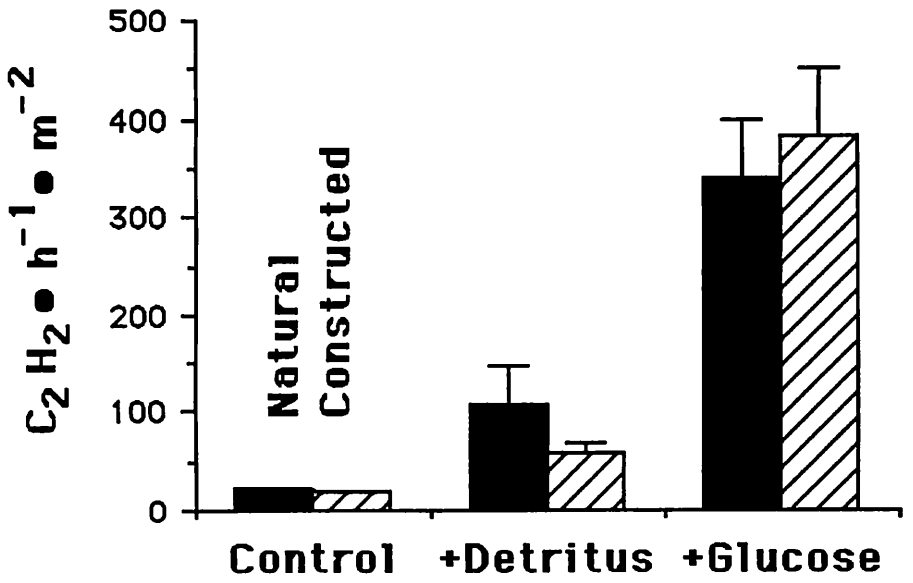

Figure 2. Effect of detritus and glucose additions on nitrogen fixation rates in natural and constructed marshes. Data are means ± SE.

produced underground. More substantial stimulation of nitrogen-fixation rates was obtained in both marshes when glucose was added to the cores (Figure 2). This implies that the more refractory organic compounds in detritus cannot be used by the nitrogen fixers and must be decomposed into simpler compounds. The positive response of the microbial community indicates that it might be possible to manipulate nitrogen cycling by adding organic matter. Results from San Diego Bay were similar to those in Texas (Lindau and Hossner, 1981) and North Carolina (Craft et al., 1986, 1988); for each comparison, the constructed marsh had lower levels of nutrients and organic carbon than the adjacent natural marsh (Table 3).

Four years after construction and planting with *Spartina*, the constructed marsh had not developed nutrient pools comparable to those of the reference marsh. The study period was not long enough to see significant increases in nitrogen and extractable ammonium in pore water, or in total nitrogen and percentage of organic carbon in sediment. The changes in nitrogen fixation in response to amendments with organic matter indicated that the process is energy limited. The mineralization experiment showed that microbial activity was similar in both marshes. Therefore, low nutrient levels in the constructed marsh could result from the poorer contribution of organic matter provided by sparse stands of vegetation. In addition to being a source of available nitrogen to the marsh through mineralization, organic matter stimulates nitrogen fixation, thereby increasing nitrogen supplies.

The question of whether constructed wetlands will ever duplicate the nutrient cycling function of natural salt marshes remains. Our results suggest that mitigation be undertaken cautiously. Exchanging an acre of constructed wetland for an acre of natural wetland results in a net loss in wetland function. On the basis of data for Tijuana Estuary (Winfield, 1980; Covin, 1984; Covin and Zedler, 1988) and Paradise Creek Marsh, we think that nitrogen supplies and availability are close to critical limits. The likelihood that constructed wetlands will have even lower pools of nitrogen and organic matter means that the nutritional status and productivity of the vegetation in man-made salt marshes will be even more severely limited. These predictions are borne out by the lower biomass and foliar nitrogen concentrations of *S. foliosa* in Connector Marsh compared with Paradise Creek Marsh. Because we do not know how long it will take for low-nutrient, inorganic soils to build up sufficient organic matter and nitrogen pools that match those of natural wetlands, the time required for constructed marshes to become functionally equivalent to natural salt marshes cannot be predicted.

Growth and Persistence of Native Plants. In 1987, after 3 years of growth, vegetation was not as well developed in the constructed marsh (Swift, 1988) as in the natural marsh. The constructed marsh had large bare areas where transplants failed to establish. Even where plant cover was high (more than 80%), plants were generally less dense (half as much) and had lower biomass (one-third to one-half as much; estimated from total stem lengths) than in the natural marsh. Soil salinity was similar for the two sites, so none of these effects could be attributed to salt. An experimental transplanting in 1987 (by K. Swift) indicated that bare areas were not due to inadequate soil conditions because no transplants died. Thus, the rough handling of the original transplanting material may have reduced establishment, and the constructed

Table 3. Organic Carbon and Total Nitrogen in Natural and Constructed Marshes of Texas

	Organic Carbon (%)		Total Nitrogen (mg/kg dry weight)	
Location (age in years)	Natural	Constructed	Natural	Constructed
Texas (1)	0.3–1.12	0.13	227–588	95
North Carolina (10–15)	0.6–8.6	0.6–1.8	364–1680	322–924
San Diego Bay (4)	2.0–2.5	0.1–1.1	1740–2270	870–960

Texas data from Lindau and Hossner, 1981; North Carolina data from Craft et al. 1988. Years are times between marsh construction and sampling.

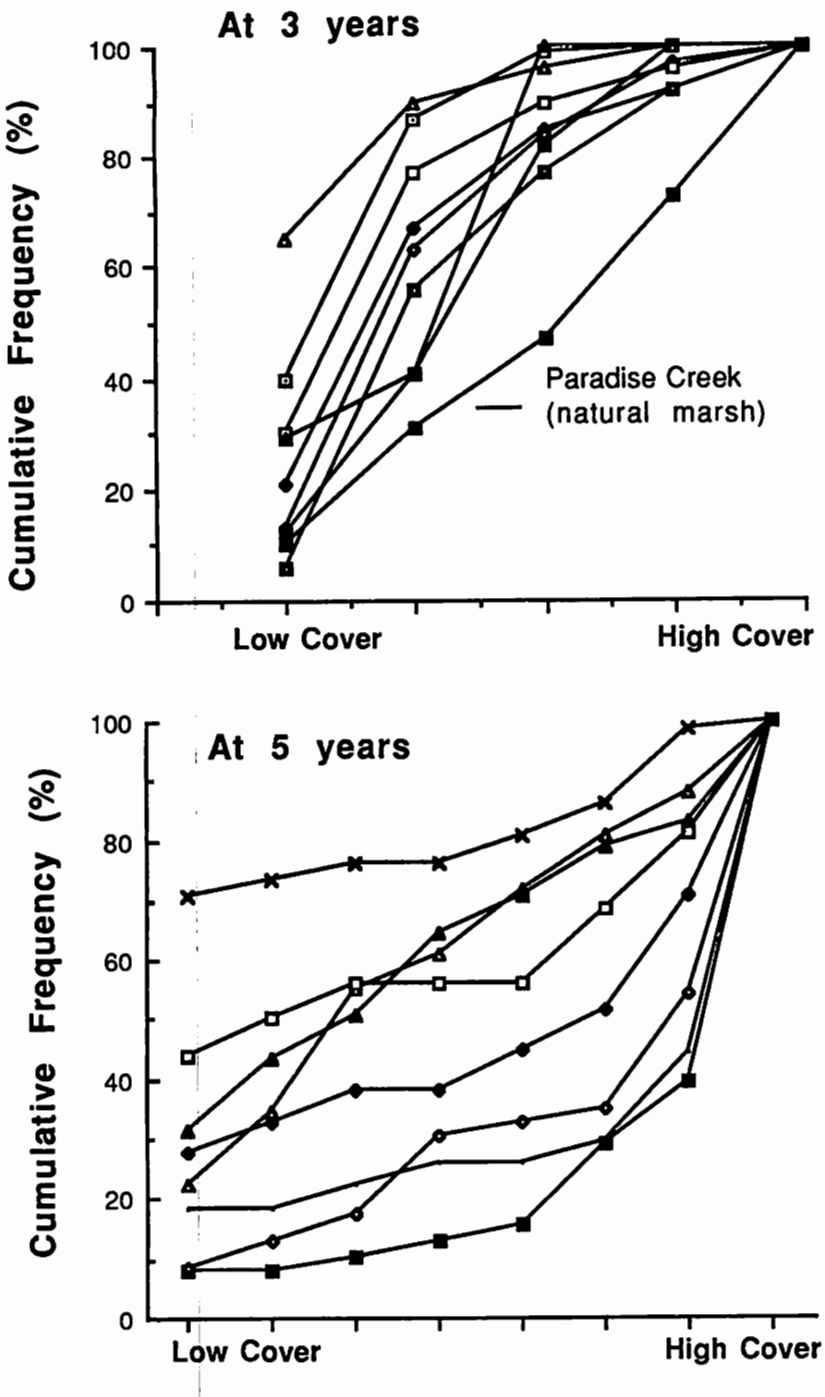

Figure 3. Cover of cordgrass (*Spartina foliosa*) in the north and south islands of the Connector Marsh (constructed) at ages 3 and 5 years. Data are cumulative histograms for the percentage of 4-m intervals with low to high cover, with one line for each island. Convex curves (at 3 yr) result from large numbers of intervals with little plant cover; concave curves (at 5 yr) result when most intervals have high cover. Data for Paradise Creek (natural marsh) were collected at the same time as the 3-year sample of the constructed marsh.

marsh should eventually achieve high plant cover through vegetative expansion of cordgrass that survived the first 3 years.

A resurvey of the same sampling sites in 1989 indicated substantial improvement in plant cover (Figure 3). Cordgrass densities were similar at the sampling stations in the constructed and natural marshes; however, biomass above ground, as indicated by total stem length, was still lower at Connector Marsh (Table 4).

Food chain support. The availability of foods for marsh carnivores was examined as a measure of food chain support. Rutherford (1989) emphasized small invertebrates (crabs, amphipods, insects), which are potential food items for shorebirds and the endangered light-footed clapper rail.

More species of invertebrates were found in litter bags placed in the lower elevation at Paradise Creek Marsh than in Connector Marsh. The species lists for the two sites were only 46% similar. The most common species was a larval dipteran, *Pericoma* sp. (35% of all animals trapped). In addition, a native anemone, *Diadumene franciscana*, was found only in the natural marsh. In the constructed marsh, significantly more *Hemigrapsus* crabs were found at all sites sampled.

Densities of epibenthic invertebrates were substantially higher in the natural marsh. Of 43,531 invertebrates trapped over eight sampling periods, 31,957 (73.4%) were in the natural marsh. The dominant species, *Pericoma* sp., was significantly more abundant (up to 9x) in the natural marsh. There was a linkage with plant biomass; natural marsh areas with 80–100% cover of cordgrass supported 9–62% more invertebrates than areas with 0–20% cover (steep banks).

Sampling for invertebrates indicated the presence of an exotic mussel, *Musculista senhousia*, which has successfuliy invaded both San Diego Bay and Mission Bay and displaced native bivalves in subtidal areas. It thus represents a serious threat to the native benthic community. Of the 55 individuals trapped in litter bags, 47 were in the constructed marsh.

Discussion

The presence and efficient cycling of nutrients are central to the development and maintenance of marshes, whether the marshes are natural or constructed. Of particular

Table 4. Comparisons of Density and Total Stem Length of Cordgrass in Constructed and Natural Salt Marshes of San Diego Bay

	Density (No./m^2)	Total Stem Length (m/m^2)
Constructed Marshes		
Connector Marsh NI1	173 (38)	63 (15)
Connector Marsh NI2	137 (3)	70 (2)
Connector Marsh NI3	133 (12)	87 (15)
Connector Marsh NI4	153 (15)	48 (8)
Natural Marshes		
Paradise Creek PC1	140 (27)	102 (22)
Paradise Creek PC2	187 (26)	129 (14)
Paradise Creek PC3	193 (35)	131 (29)

Data are means (and S.E.). NI1–NI4 and PC1–PC3 indicate sampling sites.

importance in coastal marine environments is nitrogen (Valiela and Teal, 1979; Covin and Zedler, 1988; Howarth, 1988). Nitrogen levels and availability affect marsh plant productivity, standing biomass, diversity, morphology, reproductive potential, abundance of plant species, and nitrogen content of plant biomass (Valiela, 1983). These features in turn influence the invertebrate and vertebrate animals of the wetlands. Inadequate supplies of nitrogen are likely to affect total wetland functioning, by altering the basic ecosystem processes of primary productivity, decomposition, and food chain support.

Summary and Recommendations

Soil nitrogen pools were lower in a 4-year-old constructed salt marsh than in an adjacent natural marsh of San Diego Bay. Aboveground biomass and foliar nitrogen content of *S. foliosa* were both lower for the constructed marsh. Soil organic carbon, which was highly positively correlated with total nitrogen, was also lower in the constructed marsh. Nitrogen-fixation rates were higher for the natural marsh in surface soils (1-cm depth) but not in the rhizosphere (10-cm depth). Experimental additions of organic matter increased nitrogen-fixation rates substantially for both constructed and natural marsh soils, with glucose stimulating greater increases than *S. foliosa* detritus (roots and rhizomes). In comparison with natural marshes studied elsewhere, the San Diego Bay sites have low nitrogen pools and little soil organic carbon. Nitrogen mineralization rates (in situ incubations) were high in both marshes studied. The low nitrogen pools reflect low tidal import and infrequent streamflow influxes and, possibly, high nitrogen demands of vegetation stressed by hypersaline soils.

Although 4 years was not enough time for this constructed salt marsh to duplicate the functions of natural marshes, our results suggest that augmenting soil organic matter before transplantation could accelerate the rate of ecosystem development.

Cooperating Organizations

California Coastal Commission
California Department of Parks and Recreation
California Department of Transportation
California State Coastal Conservancy
California State Department of Fish and Game
Los Peñosquitos Lagoon Foundation
National Marine Fisheries Service, Southwest Region
National Oceanic and Atmospheric Administration, Ocean and Coastal Resources Management, Marine and Estuarine Management Division
U.S. Army Corps of Engineers
U.S. Environmental Protection Agency Regional Office
U.S. Fish and Wildlife Service

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Seagrass Revegetation: Physiological and Environmental Criteria for Successful Transplanting

San Francisco State University
R/CZ-83
Project Initiated: October 1, 1987
Project Completed: December 31, 1989

Michael Josselyn and Randall S. Alberte

Introduction

Although seagrass meadows are important and often critical components of nearshore ecosystems (Mann, 1982; Fonseca and Kenworthy, 1987; Phillips, 1984; Thayer et al., 1984; Orth and Moore, 1988), they are under increasing pressure from human development of the coastal zone, which has reduced their abundance and distribution in estuarine environments throughout the world. The ecological importance of seagrass meadows has prompted numerous efforts to mitigate the damage to seagrass populations caused by such development, which usually involve restorative transplanting of seagrasses into the affected area or into a designated mitigation site that may not be near the site of disturbance. Guidelines for transplanting seagrasses have been based on practical experience gained through individual case studies and economic considerations, because unequivocal experimental evidence evaluating various transplant methods has not been available. Although the plug method (which leaves the sediment environment surrounding the roots intact) appears to result in higher rates of plant survival (Phillips, 1980a, 1980b; Lewis, 1987), the bare-root technique (which involves the removal of plants from their native sediments and placement directly into the often anoxic sediment at the transplant site) appears to be the method of choice, primarily for economic reasons (Fonseca et al., 1985).

In addition to proper transplanting technique, the success of any revegetation effort requires a physical environment that will support long-term growth. The availability of light is important for seagrass survival (Backman and Barilotti, 1976; Orth and Moore, 1983; Dennison and Alberte, 1985, 1986; Dennison, 1987); however, light requirements are not yet understood well enough that availability of light can be used as a reliable predictor of environmental suitability.

Objectives

The overall objectives of this project were (1) to help define the physiological and environmental factors needed for successful transplanting of the eelgrass *Zostera marina* into anoxic sediments characteristic of most estuarine environments and (2) to construct a predictive model that can be used to help identify suitable sites for seagrass revegetation and mitigation efforts.

The objective for the first year was to define the most successful transplanting methods using mesocosms and controlled experimental conditions. Also, we used light, growth, and physiological data collected from plants in the mesocosms to define minimum light requirements for successful growth of eelgrass in San Francisco Bay.

The objective for the second year was to evaluate the predicted light requirements for eelgrass growth generated in the first year. The transplant method found most successful in the mesocosm experiments was used to plant seagrasses along a depth gradient at two sites in San Francisco Bay that were characterized by different amounts of light availability. These plants were then followed over a full annual cycle to compare rates of survival, growth, and productivity at different depths to the model's predictions.

Results

The study sites used for this project were located in central San Francisco Bay (Figure 1). All field work and laboratory analyses were conducted from San Francisco State University's field station, the Romberg Tiburon Center for Environmental Studies (RTCES).

Mesocosm Transplant Experiment. Intact plugs of sediment containing *Z. marina* were collected from an existing eelgrass meadow at Pt. Molate in central San Francisco Bay using the rhizosphere core method (Dennison and Alberte, 1982); these plugs were placed into 1-gallon (4.4-l) plastic flower pots lined with polyethylene bags. The plugs were transported to the RTCES within 2 hours of collection, where they were prepared for experimental manipulations in mesocosms plumbed with flowing water from San Francisco Bay.

Half of the plugs were placed directly into the mesocosms, and the other half were prepared as units for bare-root planting by removing plugs from the pots and gently washing the surrounding native sediment from the roots. Half of the bare-root planting units were held in a sediment-free (hydroponic) condition for 2 weeks

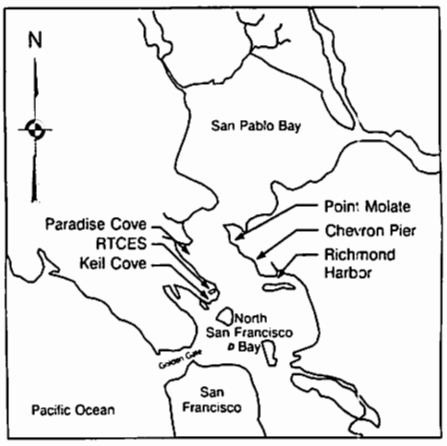


Figure 1. Locations for laboratory and field studies for the seagrass restoration project.

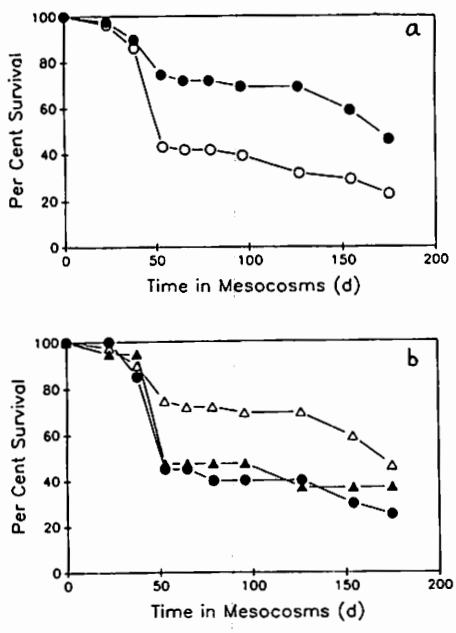


Figure 2. (a) Pattern of survival in bare-root (○) and sediment-plug treatments (high-light only, ●). All bare-root subtreatments found to be not significantly different were combined for this comparison. (b) Pattern of survival among plug treatments grown at 100% irradiance (△); 30% irradiance but conditioned for 14 days first at 100% irradiance (▲); and 30% irradiance without high-light conditioning (●). d = days.

while the others were placed directly into 1-gallon (4.4-l) flower pots containing either anoxic sediments free of eelgrass collected at the Pt. Molate donor site or oxidized beach sand collected from Keil Cove on the Tiburon Peninsula. Within each treatment group, the plants were segregated equally into shaded mesocosms (30% ambient irradiance) and unshaded mesocosms (100% ambient irradiance). Water temperature and light attenuation were measured in the mesocosms weekly, and the plants were monitored every 14 days for growth and survival. Compared with the results of other treatments, the results of the bare-root treatment showed no statistically significant effects on transplant survival; the overall survival rate of all bare-root transplants was about 20% for 6 months. However, survival rates of transplants subjected to the sediment-core treatment were higher than 50% when the transplants were maintained in the unshaded light environment (Figure 2). Reducing the light environment to 30% of ambient light caused the survival rate of sediment-plug treatments to decrease approximately to the survival rate of the bare-root transplants. In conclusion, no specific combination of bare-root transplant treatments (sediment type, light environment, or hydroponic conditioning) permitted survival rates to approach those of the sediment-plug treatments at ambient irradiance. Furthermore, shading reduced the survival rate of sediment-plug transplants to that of the bare-root treatments.

Metabolic Rate Measurements. There were no significant effects of light environment or transplant technique on rates of light-saturated photosynthesis, the irradiance required to saturate photosynthesis or dark respiration (Figure 3). The response among plants was somewhat variable but could not be related to any effects of experimental treatment and might represent natural variability among the plants in this population. Accordingly, the data were pooled to determine average rates of net photosynthesis and respiration for use in calculating whole-plant carbon budgets.

Modeling Light Requirements. On the basis of the mean ratio of photosynthesis to respiration reported for these data and carbon-budget calculations as performed by Zimmerman et al. (1989), eelgrass plants from San Francisco Bay should be able to meet their minimum daily carbon requirements with approximately 4 hours of irradiance-saturated photosynthesis (H_{sat}) each day. However, light-attenuation data collected in the mesocosms suggest that plants in the shaded mesocosm may have been severely light-limited at times: H_{sat} periods calculated from attenuation coefficients were frequently below the 4-hour requirement (Figure 4). However, plants in the unshaded mesocosms were exposed to much longer H_{sat} periods and consequently were not light-limited. These results are in agreement with the mesocosm survival patterns discussed earlier.

The Light Environment of San Francisco Bay and the Distribution of Natural Eelgrass Populations. Light-attenuation coefficients measured weekly at five sites in central San Francisco Bay showed a high degree of temporal variability (Figure 5). In general, San Francisco Bay appears to be characterized by large spatial and temporal variations

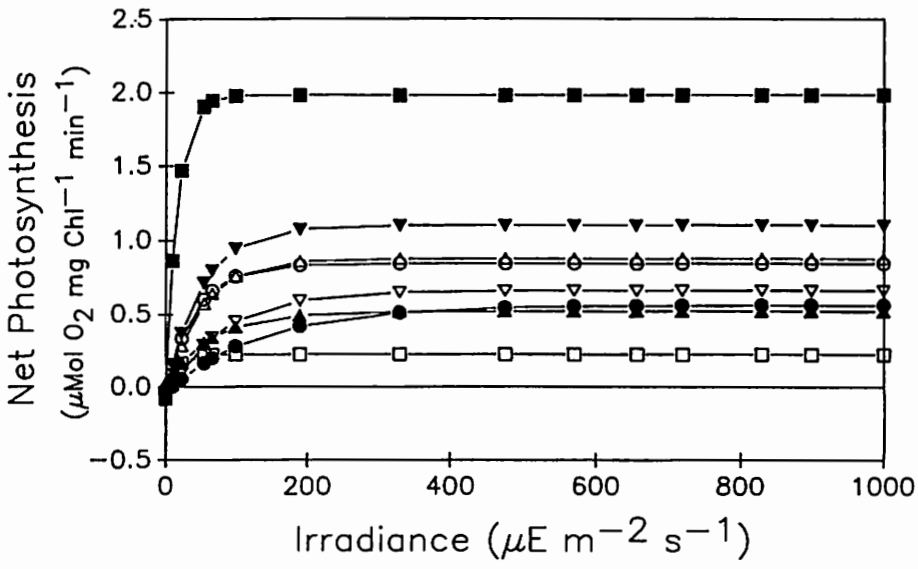
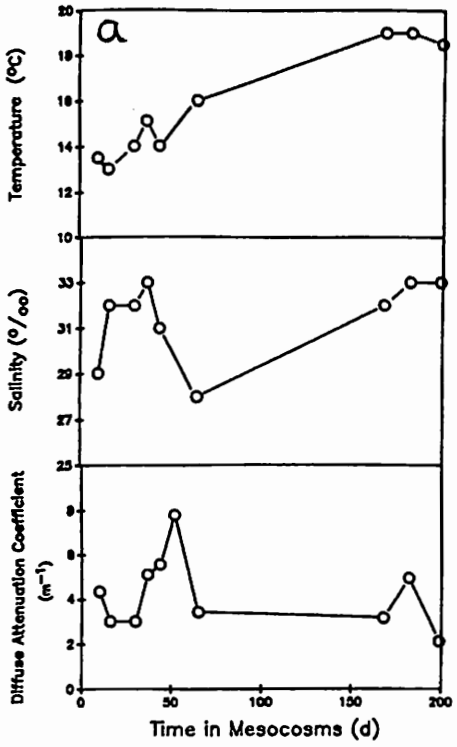


Figure 3. Photosynthesis vs. irradiance (*P* vs. *I*) curves generated from plants in shaded (filled symbols) and unshaded (open symbols) mesocosms. In each case, the data were fit directly to the exponential function of Platt and Gallegos (1980), and r^2 values for each fit were above 0.95.



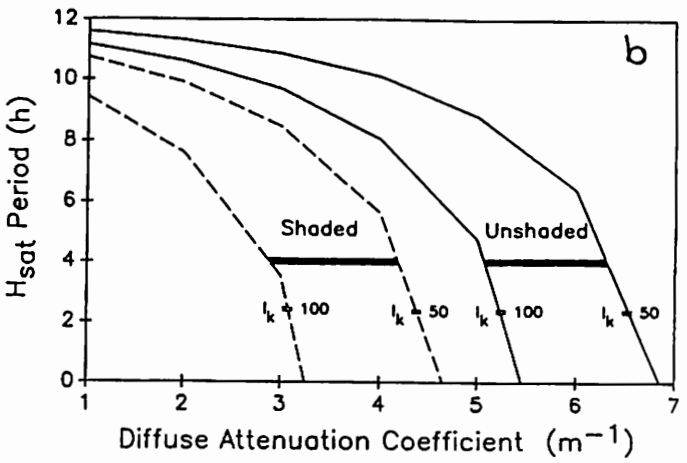


Figure 4. (a) Time series of water temperature, salinity, and diffuse attenuation coefficient in the mesocosms during the transplant experiment. (b) The effect of diffuse attenuation coefficient (k) on estimates of H_{sat} in the shaded (30% ambient irradiance) and unshaded (100% ambient irradiance) mesocosms at 0.5 m depth. Upper and lower bounds for each mesocosm were drawn to include the effect of uncertainty in the value of I_k (irradiance for any given diffuse attenuation coefficient) on H_{sat} estimates. Solid bars at H_{sat} = 4 hours indicate the range of diffuse attenuation coefficients that permitted photosynthesis to meet daily carbon demand in the two light environments. d = days; H_{sat} = irradiance-saturated photosynthesis; h = hours.

in diffuse attenuation coefficient, although this coefficient is almost always high enough to limit the euphotic zone in the Bay to the upper 3 m (Figure 6). As a result, H_{sat} periods adequate for supporting eelgrass growth were limited to shallower depths, because 4-hour H_{sat} periods extended only 2 m into the water column. (This calculation is based on mean values of attenuation coefficient found in this study.)

The temporal and spatial variation in light availability documented above appears to have implications for eelgrass distribution in the Bay as well. Plants were found growing to greater depths at sites with the clearest water, and these depth limits were closer to the theoretical H_{sat} limits than were the depth limits at more turbid sites (as indicated by relatively low attenuation coefficients (Figures 7 and 8).

Results presented to this point represent our research efforts completed during the first year of the project. Analysis of the complete data set can be found in Zimmerman et al. (1991) and Raguzzini et al. (in prep).

Field Transplant Experiment. During the second year of the study,

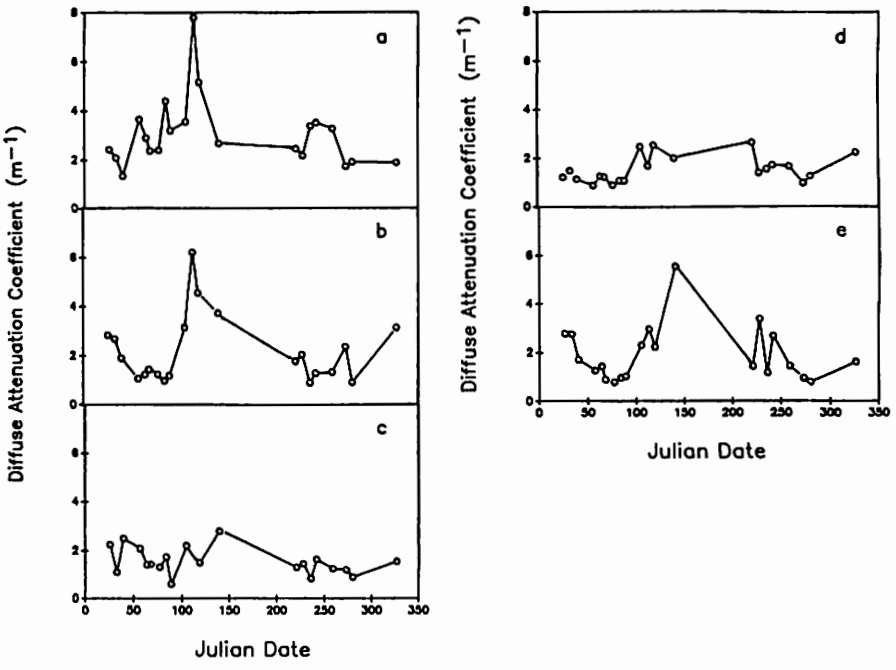


Figure 5. Time series of diffuse attenuation coefficients at the field sites: (a) Paradise Cove, (b) Pt. Molate, (c) Keil cove, (d) Richmond Harbor, and (e) Chevron Pier.

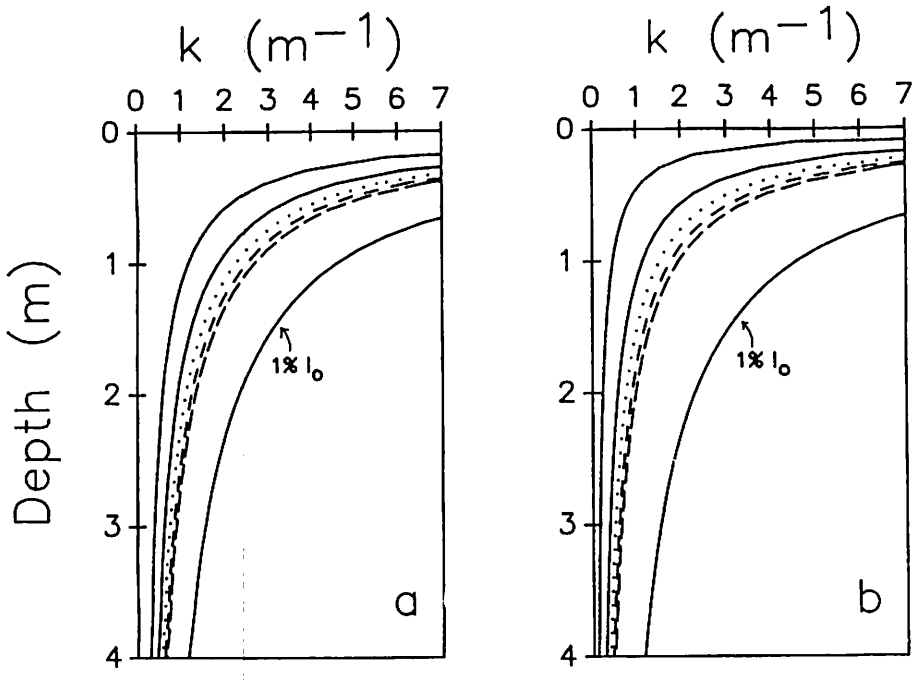


Figure 6. Distribution of $_{sat}$ plotted in parameter space defined by irradiance (I) of a diffuse attenuation coefficient (k) and depth. (a) I_k = 50 µE m^{-2} s^{-1}. (b) I_k = 100 µE m^{-2} s^{-1}. The depth of the euphotic zone (1% I_o) is included for comparison. Double-dash line is for H_{sat} = 2 hours; single-dash line, H_{sat} = 4 hours; dotted line, H_{sat} = 6 hours; double-dash, single-dot line, H_{sat} = 8 hours; and double-dash, double-dot line, H_{sat} = 10 hours.

to test the calculated H_{sat} requirement of 4 hours, we transplanted eelgrass using the rhizosphere-core technique into plots at Keil Cove and Paradise Cove on the Tiburon Peninsula. Cores were placed into three transects at each site, running along a depth gradient from 2 m to 0 m MLLW. Plants along the depth gradient were sampled every 3 months for survival, growth rates, metabolic activity, and carbohydrate content. The sampling scheduled for February 1990 completed a full annual cycle. Although we have only performed preliminary analysis of the data, a few patterns are emerging. The quantum efficiency of photosynthesis (α) and the maximum rate of photosynthesis (P_m) appear to respond to seasonal changes in light availability in a pattern typical of light adaptation. Quantum efficiency was higher and P_m was lower during the winter low-light period than during the summer high-light period.

Growth rates also indicate a seasonal pattern: They are highest in the summer at both sites. We also found that growth rates are lower at

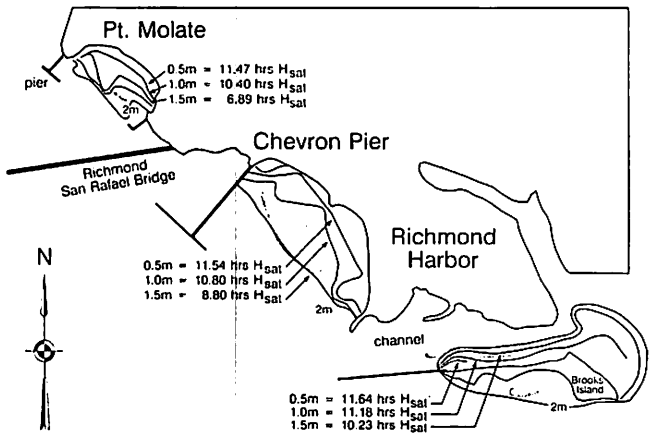


Figure 7. Eelgrass distributions (shaded area) at field sites along the eastern shoreline of San Francisco Bay, taken from Wyllie-Echeverria and Rutten (1989) and related to calculated H_{sat} values from field measurements of light attenuation. Depth contours overlaid on these distribution maps were taken from U.S. Department of Commerce (NOAA) navigational chart #18649, dated 18 October 1986.

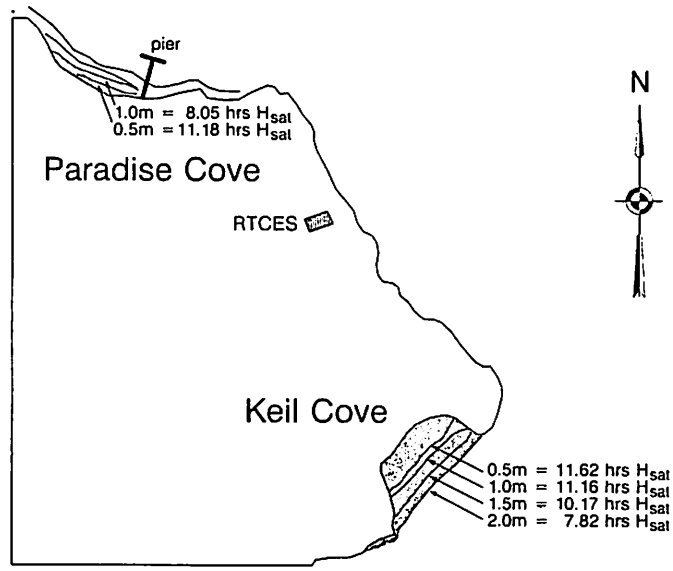


Figure 8. Eelgrass distributions (shaded area) at field sites along the western shoreline of San Francisco Bay, taken from Wyllie-Echeverria and Rutten (1989) and related to calculated H_{sat} values from field measurements of light attenuation. Depth contours overlaid on these distribution maps were taken from U.S. Department of Commerce (NOAA) navigational chart #18649, dated 18 October 1986. RTCES = Romberg Tiburon Center for Enviromental Studies.

Paradise Cove (the more turbid site) than at Keil Cove. However, the data do not yet show a significant trend relating to the depth gradient at either site. Analysis of these data is continuing. We anticipate that a manuscript describing the results of these studies will be ready for submission to a peer-reviewed journal by September 1990.

Cooperating Organizations

California State Department of Fish and Game
National Marine Fisheries Service
Romberg Tiburon Center for Environmental Studies
The Winifred and Harry B. Allen Foundation
U.S. Army Corps of Engineers

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Management Models of Wetland Wastewater Treatment Systems

Humboldt State University
R/CZ-84
Project Initiated: October 1, 1988
Project Completed: September 30, 1990

Brad A. Finney and Robert Willis

Introduction

During the past decade, it has been shown that using freshwater wetlands for the treatment or polishing of municipal wastewater is a reliable and cost-effective alternative to other methods (Gearheart and Finney, 1982; Gearheart et al., 1984a). Field studies have shown that wetlands have several distinct advantages over conventional systems used to treat waste water. For example, wetland systems are relatively insensitive to hydraulic or organic shock (pulse) loading. They provide significant removal of heavy metals and other toxic compounds because of the high adsorptivity of organic soils and emergent plant uptake (Larson, 1960; Kardos, 1967; Gearheart and Finney, 1986). Wetland soils also have low hydraulic conductivity, reducing problems of seepage and groundwater contamination problems that can occur in systems that use oxidation ponds. Although wetlands have an important ecological and recreational value, they have a relatively low economic value. The low cost of wetland disposal as tertiary treatment and the low value of land are important considerations for rural areas or areas with seasonally low populations that might not be able to afford more expensive methods of waste treatment (Dixon and Kadlec, 1975). In addition, wetland systems provide significant removal of pathogens and provide for resource recovery through biomass fermentation (Gearheart et al., 1984a).

The use of wetlands for wastewater treatment is rapidly evolving from an innovative technology to an accepted alternative (EPA, 1985). In 1984, for example, more than 1000 citations of published information on wetlands waste treatment systems appeared in the literature (Knight, 1987). Design information has also been summarized by Chan et al. (1982) and Hammer and Kadlec (1983). In California and other coastal states, the success of demonstration systems has resulted in a number of full-scale operational systems. Controlled discharges can enhance the productivity and therefore the commercial and recreational value of receiving waters. Wetland systems also are valuable as educational, recreational, and animal habitat sites.

Although significant progress has been made in understanding the hydraulics and nutrient cycling that occur in wetlands, the optimal management of wetlands and the evaluation of the economic, environmental, hydraulic, and waste treatment trade-offs associated with various management strategies have not been addressed in field or research studies.

Project Objectives

The overriding objective of this research is the development of management models for wetland wastewater treatment systems. The management models consist of simulation and optimization models that will be used to evaluate waste treatment performance; determine waste control stategies; and identify environmental trade-offs for a range of economic, environmental, and hydrological conditions commonly encountered in freshwater marshes.

During the first year of the study, hydrodynamic and water quality simulation models for wetland systems were developed. Initial calibration and validation of the models were done by using the City of Arcata's data base on its full-scale wetland treatment system.

During the second year of the study, the specific objective was to develop management models for wetland treatment of wastewater that identify trade-offs between environmental and economic conditions and determine waste treatment control strategies. These management models used the simulation models developed in the first year of the study and focused on optimal hydraulic management of a wetland system to maximize pathogen removal. Specific tasks required to accomplish this objective included (1) identification of the mechanisms through which pathogen removal occurs in a wetland system, (2) devlopment of kinetic models that relate wetland operational conditions to pathogen removal and the incorporation of these kinetic models into simulation models of the Arcata wetland system, and (3) identification of optimization algorithms appropriate for the determination of management strategies for optimal pathogen removal in wetland wastewater treatment systems.

Summary of Progress

Most of the second-year goals have been met. The following was accomplished to meet these goals: (1) The literature was reviewed to determine mechanisms through which pathogen removal and inactivation may occur in a wetland system and how these mechanisms are related to water quality and waste treatment control strategies. (2) The mechanisms identified were used to develop kinetic relationships that describe the data for coliphage removal and inactivation obtained from a 6-month study of the Arcata pilot waste treatment system. (3) Specific objectives and constraints for incorporation into the management optimization models and appropriate mathematical programming algorithms for solution of the proposed optimization problems were determined.

Literature Review of Pathogen Removal in Wastewater. The ability of wetlands to remove human enteric bacteria is well documented. Spangler et al. (1976) found a 90 to

99.7% reduction in coliform bacteria in pilot plant studies. Dinges (1979) reported coliform removal rates of 98% in a similar pilot plant and 94% in a full-scale hyacinth treatment system. Reed et al. (1988) summarized the performance of four wetland wastewater treatment systems, including the Arcata Marsh system. In all four systems the annual coliform removal rates were more than 90%.

Gearheart et al. (1984b) found that removal rates in the Arcata pilot wetland treatment system were about 86%. Reduced hydraulic loading increased removal rates. This may account for the reduced removal efficiencies during the winter reported by Reed et al. (1988). Other studies of the Arcata pilot treatment system have shown an average removal of enteric *Salmonella* sp. of 95%.

Several mechanisms may contribute to the inactivation or removal of viruses and indicator organisms in a wetland treatment system. Physical factors include photooxidation, adsorption, flocculation, coagulation, sedimentation, and water temperature. Physiochemical factors include osmotic effects, pH, chemical toxicity, and redox potential. Biochemical-biological factors include nutrient levels, presence of organic substances, predators, bacteriophages, algae, and the presence of fecal matter. Many of these removal mechanisms may be insignificant.

Determining the dominant removal and inactivation mechanisms in wetland systems is difficult because of the lack of studies designed to determine such mechanisms and the associated removal rates. Gearheart et al. (1986) showed that the removal of total and fecal coliforms depends on aquatic vegetation. The study compared harvested vs. unharvested cells at the Arcata pilot treatment system. Unharvested cells consistently showed a greater removal of coliforms. The biofilm associated with the emergent vegetation may adsorb and inactivate coliforms. Coliforms may adsorb to suspended solids (SS) and settle out of the water column, with emergent vegetation contributing to the stabilization of the settled SS. In addition, Gearheart et al. (1984) showed that the efficiency of pathogen removal depended on hydraulic loading.

In contrast to studies of wetland treatment systems, pathogen removal in conventional waste treatment systems and other natural systems has been studied extensively. Obvious similarities between these systems and wetland treatment systems can be used to infer possible pathogen removal and inactivation mechanisms and rates in a wetland treatment system. Reed et al. (1988) assert that mechanisms of pathogen removal in a wetland system are similar to those in conventional pond systems, with a possible increase in filtration capacity.

Klock (1971) states that coliform inactivation in wastewater lagoons is associated with an endogenous metabolism and the hostile environment of the wastewater. Temperature and pH were incorporated into an Arrhenius relationship to describe the viral kinetics observed in field studies. Results of the study were compared with those from other research, and removal rates were comparable.

Sarikaya and Saatci (1988) related bacterial die-off to the effect of pond depth on ultraviolet inactivation. Die-off rate constants were inversely proportional to pond depths. Die-off rate constants were inversely proportional to pond depths (Sarikaya and Saatci, 1987). Kinetic models were used in completely mixed and dispersed-flow pond models to determine optimum pond depths on the basis of minimum cost objectives for a given bacterial removal efficiency.

Polprast and Hoang (1983) studied two anaerobic filters with different specific surface areas. They found that first-order kinetics adequately described the efficiencies of the two filters for removing pathogens. The removal rates were 0.92 day^{-1} for fecal coliforms and 0.54 day^{-1} for bacteriophages. An interesting result of the study was the comparable removal rates of the two systems under various hydraulic loadings. Because adsorption is assumed to be proportional to specific surface area, it was concluded that the removal and inactivation of coliforms and bacteriophages were more strongly influenced by the effects of water quality and filtration than by adsorption.

Polprast et al. (1983) studied the effect of algal concentration, organic loading, ultraviolet light, temperature, and flow regimen on bacterial die-off kinetics in laboratory and full-scale waste stabilization ponds. A kinetic relationship was developed that incorporated the mechanisms.

Although predation (Gophen, 1985), enzymatic inactivation (Patti et al., 1987), chemical toxicity, and a host of other removal and inactivation mechanisms are reported in the literature, adsorption, sedimentation, and ultraviolet inactivation are the main mechanisms cited. Factors often reported as affecting the efficiency of these mechanisms include temperature, algae, amount of dissolved oxygen, pH, biological oxygen demand (BOD), SS, detention time, and water depth (Mancini, 1978).

Although several models relating viral kinetics to one or more of these factors have been proposed (Mancini, 1978; Polprast and Hoang, 1983; Polprast et al., 1983; Sarikaya and Saatci, 1988), many authors have emphasized the need to improve existing models of pathogen decay (Bowles, 1979; Finney and Middlebrooks, 1980).

Kinetic Model of Virus Removal in the Arcata Wetland System. Kinetic relationships reported in the literature were reviewed and analyzed in order to determine which relationships best described data obtained from the 1987 studies of coliphage removal in the Arcata pilot treatment system (Ives, 1987). The coliphage removal study incorporated both field and laboratory components. The field component of the 1987 study included pulse-chase experiments, establishment of steady-state coliphage plaque forming unit (PFU) profiles, and experiments with dialysis chambers to determine inactivation and removal mechanisms. Laboratory studies included determining removal efficiencies of wetland water from

various spatial locations. All components of the study involved monitoring BOD, total levels of SS (TSS), amount of dissolved oxygen, pH, conductance, and water temperature.

The hydraulic and mass-transport models for wetland systems developed during the first year of this project were used to analyze the pulse-chase data and steady-state coliphage PFU profiles. Nonlinear parameter estimation algorithms were used to determine the dispersion coefficients, D, and first-order reaction rate constants, k.

The reaction rates differed over the year of study. The differences in rate constants between study months can be attributed to seasonal variations in water temperature because other indicators of water quality remained relatively constant. An Arrhenius relationship adequately described the dependence of k on water temperature. However, first-order kinetics was unable to describe the apparent higher removal rate evident in the front end of the treatment cell. In addition, a statistically significant increase in coliphage PFUs was consistently found in an 8-ft (2.4-m) section of the marsh treatment cell. The increase corresponded to an absence of aquatic plants in the same area. These results verify results from Gearheart et al. (1986) that showed a strong correlation between removal efficiencies and emergent vegetation in a wetland system.

A strong correlation between initial levels of TSS, BOD, and virus removal efficiencies was evident in the data obtained from static samples in the laboratory. The data supported the use of an adsorption model dependent on the level of TSS, PFUs, and adsorption capacity of the SS.

From results of the data analyses, mathematical formulas for kinetics of coliphage removal in the pilot wetland treatment system were developed. The kinetic relationships include mechanisms for virus removal that depend on the density of emergent vegetation, the level of TSS and BOD, water temperature, pH, intensity of ultraviolet light, and number of coliphage PFUs. The mechanisms include adsorption of coliphages to suspended particulate matter, capture of particulate matter and free viruses by the biofilm associated with the emergent plant population, settling of particulate matter, and ultraviolet inactivation. The kinetic relationships are capable of modeling a kinetic reaction other than a first-order reaction, and as a result, good fits to the observed data were obtained.

Optimal Management Model. The kinetic relationships for removal of pathogens have several endogenous and exogenous variables. The relationships link waste management controls, environmental trade-offs, and the state of the treatment system. As a result, specific management objectives and constraints can now be formulated. For example, one management objective is

$$\min z = a \sum_i C_{c,i,t}^2$$

$$+ b \sum_i (C_{BOD,out,t} - C_{BOD,NPDES,t})^2$$

$$+ c \sum_i (C_{SS,out,t} - C_{SS,NPDES,t})^2 \ \forall t ,$$

where $C_{c,i,t}$ is the number of coliphage PFUs/100 ml at location i and time t, $C_{BOD,out,t}$ and $C_{BOD,NPDES,t}$ are predicted concentration and regulatory concentration for BOD in the effluent of the wetland system at time t, and $C_{SS,out,t}$ and $C_{NPDES,out,t}$ are the predicted concentration and regulatory concentration of SS in the effluent at time t.

Constraints include feasibility limits for all parameters, limits on the state of the system (e.g., maximum and minimum water surface elevations), and the functional relationships that relate the input and state of the system to the output of the system. Management controls include altering flow regimens, the location and type of emergent vegetation, water surface elevations, and influent flow rates. The management models will be incorporated into a nonlinear optimization routine that uses a reduced gradient solution algorithm. Solutions will be optimal in terms of the system's ability to meet or exceed discharge limits and maximize pathogen removal.

Cooperating Organizations
Arcata Wetland Task Force
City of Arcata Public Works Department
Humboldt State University Marine Science Laboratory

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Relative Holocene Sea Level Fluctuations and Vertical Crustal Movement

Humboldt State University
R/CZ-86
Project Initiated: October 1, 1988
Project Completed: September 30, 1989

Gary A. Carver and David W. Valentine, Jr.

Buried saltmarsh stratigraphy indicative of rapid submergence due to great earthquakes associated with the Cascadia Subduction Zone is found in Humboldt Bay (Vick, 1988; Vick and Carver, 1989). This study identified, mapped, and sampled buried saltmarsh stratigraphy over much of Humboldt Bay. A major emphasis was placed on identifying stratigraphic evidence of episodic, sudden subsidence. The stratigraphy is preliminarily correlated with the local structures crossing Humboldt Bay. Regional correlations to assess the seismic potential of the Cascadia Subduction Zone are in progress.

Studies investigating the buried marsh surfaces found in Humboldt Bay have been in progress since 1987. These surfaces are believed to be related to subsidence in synclines traversing Humboldt Bay. Vick (1988) has completed a Master's thesis on the stratigraphy along Mad River Slough, northern Humboldt Bay, and an abstract has been published reporting this research (Vick and Carver, 1989).

Identifying additional areas of the bay underlain by buried saltmarsh sequences was our primary goal. The Sea Grant trainee also began investigating the feasibility of using sedimentology as a tool to distinguish the sedimentary sequences related to subsidence. Several ^{14}C samples were collected from buried saltmarsh surfaces, and have been sent to a dating laboratory for analysis. When the results from ^{14}C samples are received, we hope to correlate the timing of the burial of these buried surfaces to activity on the geological structures crossing Humboldt Bay.

Theory

We used two major assumptions in our research: (1) that sea level has not changed appreciably (<1 m) in the past 3,000 years and (2) that saltmarsh surfaces are created within a narrow tidal range. The stratigraphy of repeating sequences of high marsh peats overlain by shallow water marine muds reflects sudden, episodic, tectonic subsidence. There is no evidence of a gradual transition from high-marsh to shallow-marine depositional environments; however, the transition from shallow-marine to high-marsh sedimentation is gradual (Vick, 1988). The abrupt transition between the high-marsh peats and shallow-marine bay muds supports the hypothesis of sudden episodic tectonic subsidence of the peats before the deposition of the bay muds. A sequence of buried saltmarsh surfaces with ^{14}C dates of about 250, 800, 1100, and 1300 years B.P. are found up to 3 m below present sea level in Mad River Slough (Vick, 1988; Vick and Carver, 1989). During this study, similar undated sequences were identified at other locations (Figures 1 and 2). The locations correlate well with the geologic structures, but we are hesitant to correlate the sequences between sites without using absolute dating.

Methods

One-inch cores were taken and logged in approximately 70-cm lengths, up to depths of 7 m. We noted on log sheets both the field estimates of the sediment type (e.g., silty sand, clayey silt) and the content of peat layers, shell content, and detrital content. Sites were located on Mid County Humboldt Bay series maps. The logs were used in the construction of the stratigraphic columns shown in Figures 1 and 2.

Samples from six sites were analyzed: one set from modern marsh surfaces, four from tidal wall exposures, and one from a small-bore core. Standard size-analysis procedures described in Carver's *Procedures in Sedimentology* (1971) were used. Attempts to collect continuous cores using 3-inch polyvinyl chloride (PVC) pipe failed because the sediments would not move into the PVC pipe. The problems associated with correctly locating the depth in 1-inch cores limited their use to one site, which, unfortunately, was not easily accessible.

Findings

Field Investigations. The field investigations established that several buried saltmarsh surfaces underlie large areas of the bay. At least two, and perhaps three, buried surfaces are extensive enough to relate to the large synclinal structures crossing Humboldt Bay, but absolute dating of these surfaces is needed before a correlation can be confirmed with confidence. The stratigraphy of important sites is summarized on the two maps (Figures 1 and 2). Interestingly, the Payless site (Figure 3) is located on the flank of the Eureka Anticline, which is thought to be rising at ~0.5 mm/year. At this site, a buried soil is covered by a sequence of marsh sediments. Because of its location on the flank of the growing anticline and its association with buried sediments, the Payless site could provide both information on coseismic subsidence (or uplift) events and on interseismic rebound.

Two buried surfaces identified in southern Humboldt Bay were correlated over a distance of more than 2 km. Because of the proximal location of several streams, the stratigraphy between areas is complex.

Laboratory Studies. The results of the sedimentologic investigations did not indicate an unambiguous method of distinguishing sequences related to the subsidence. We had hoped to use the changes in sediment size characteristics caused by changes in depth of deposition to identify subsidence where no buried

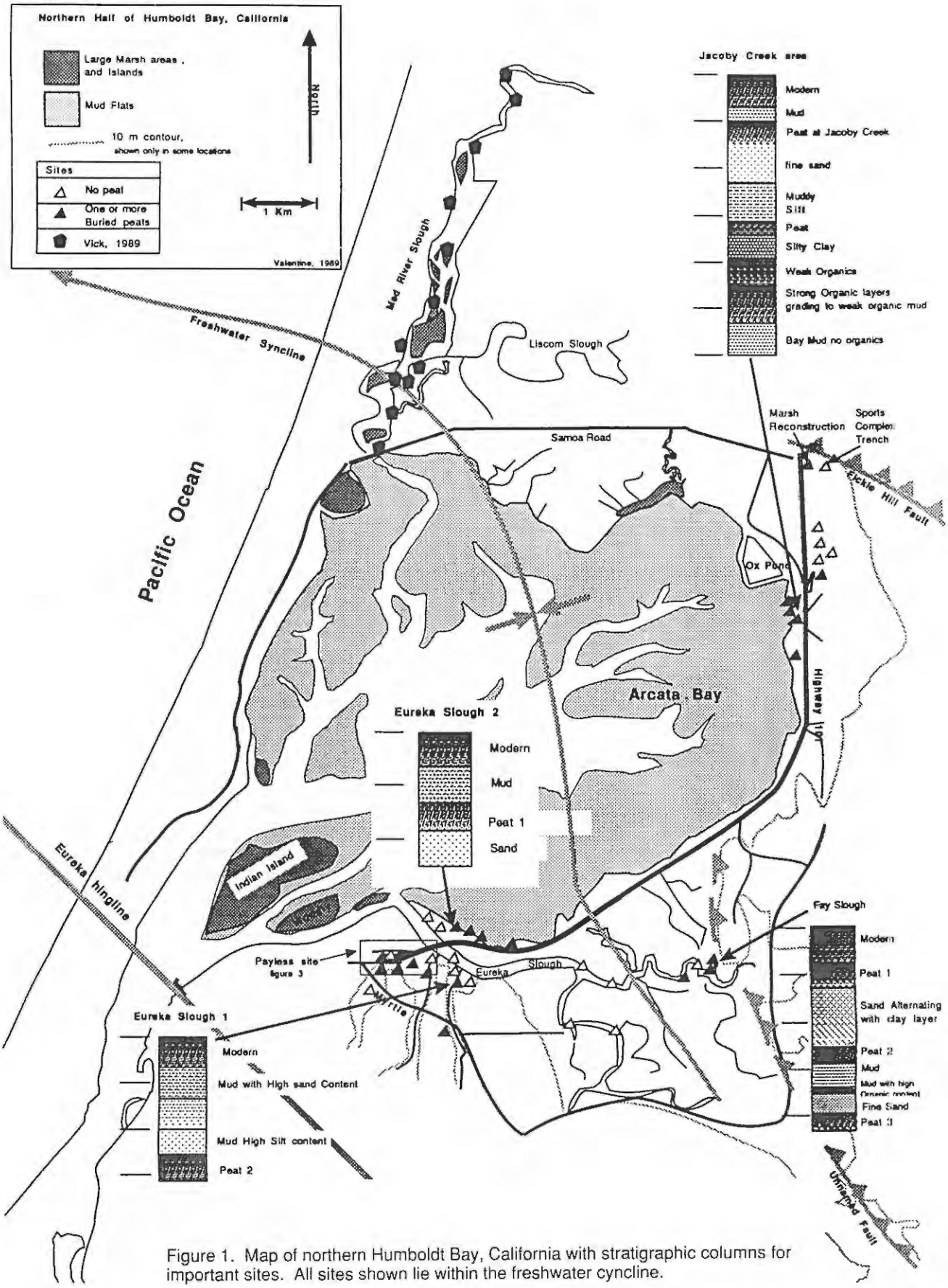

Figure 1. Map of northern Humboldt Bay, California with stratigraphic columns for important sites. All sites shown lie within the freshwater cyncline.

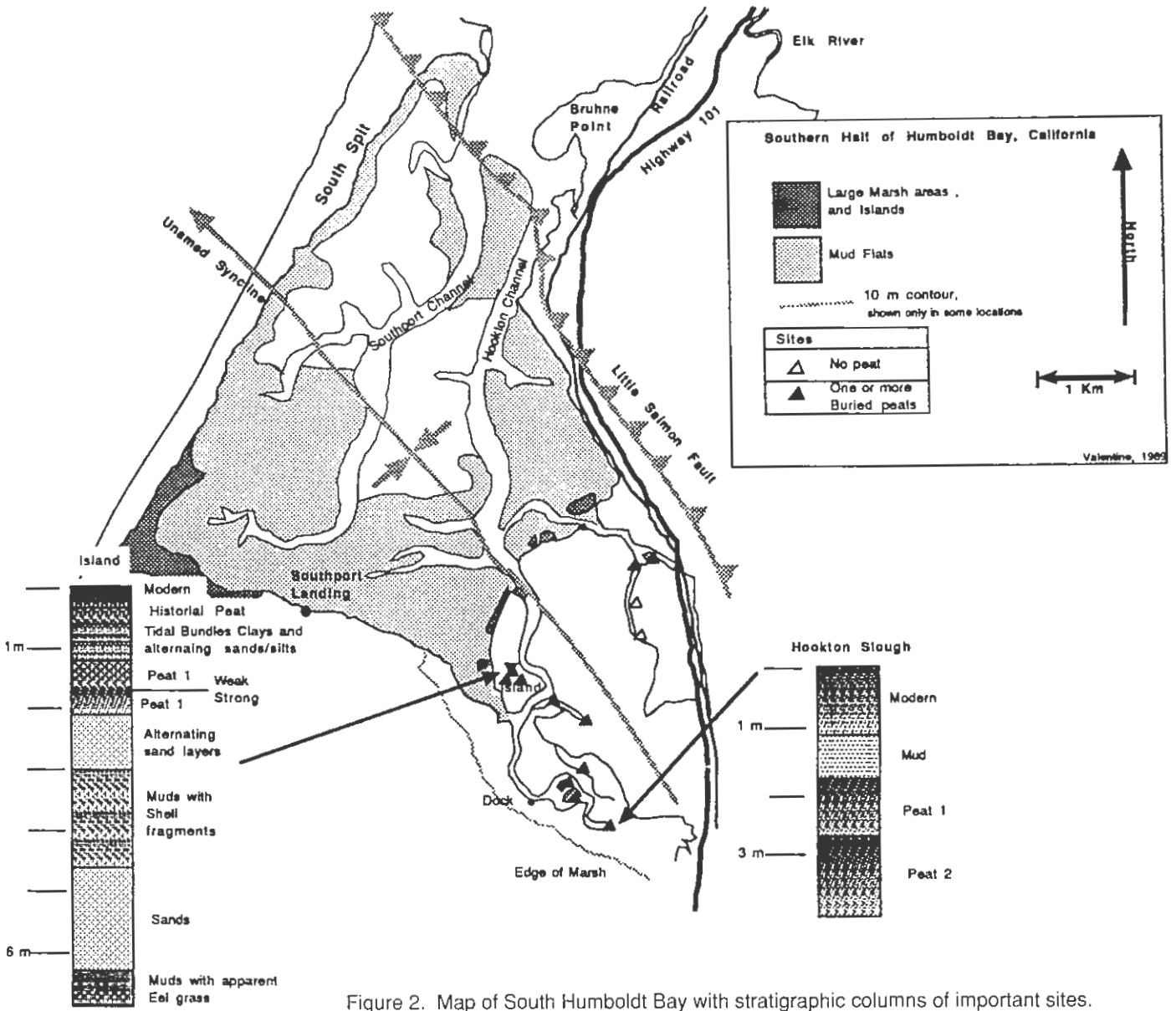

Figure 2. Map of South Humboldt Bay with stratigraphic columns of important sites. Bars along stratigraphic column represent depth in meters.

surfaces were found or where a surface was thought to be missing from a sequence. A change in silt-to-sand ratios can represent a change in depositional environment (Pejrup, 1988), but only overall changes that are not related to stratigraphy representing subsidence are seen in our data (Figure 4). Our interpretation is that the degree of subsidence is not enough to allow the use of sedimentology to distinguish the changes experienced during one tectonic event. Sedimentology can be used to distinguish long-term changes associated with sedimentation patterns in the bay and, in this study, are limited to use in reconstructions of the paleoenvironments represented in the stratigraphy found in Humboldt Bay.

Summary

The work on the subsidence features found in Humboldt Bay is important to the study of the effects of great earthquakes on the Pacific northwest. This study found more evidence that the features found in the bay are related to subsidence caused by activity on geologic structures traversing Humboldt Bay. We will be using radiocarbon dating to establish both the timing of subsidence events and the relationship of the buried saltmarsh sediments to sea-level data. The timing of subsidence events will be correlated to earthquake events known on the Little Salmon fault and other local faults to create a regional earthquake history. This regional correlation will be useful in assessing the hazard and probability of a great earthquake along the Cascadia Subduction Zone.

The sedimentology of the buried saltmarsh stratigraphy found within Humboldt Bay does not change enough over one event to be used as

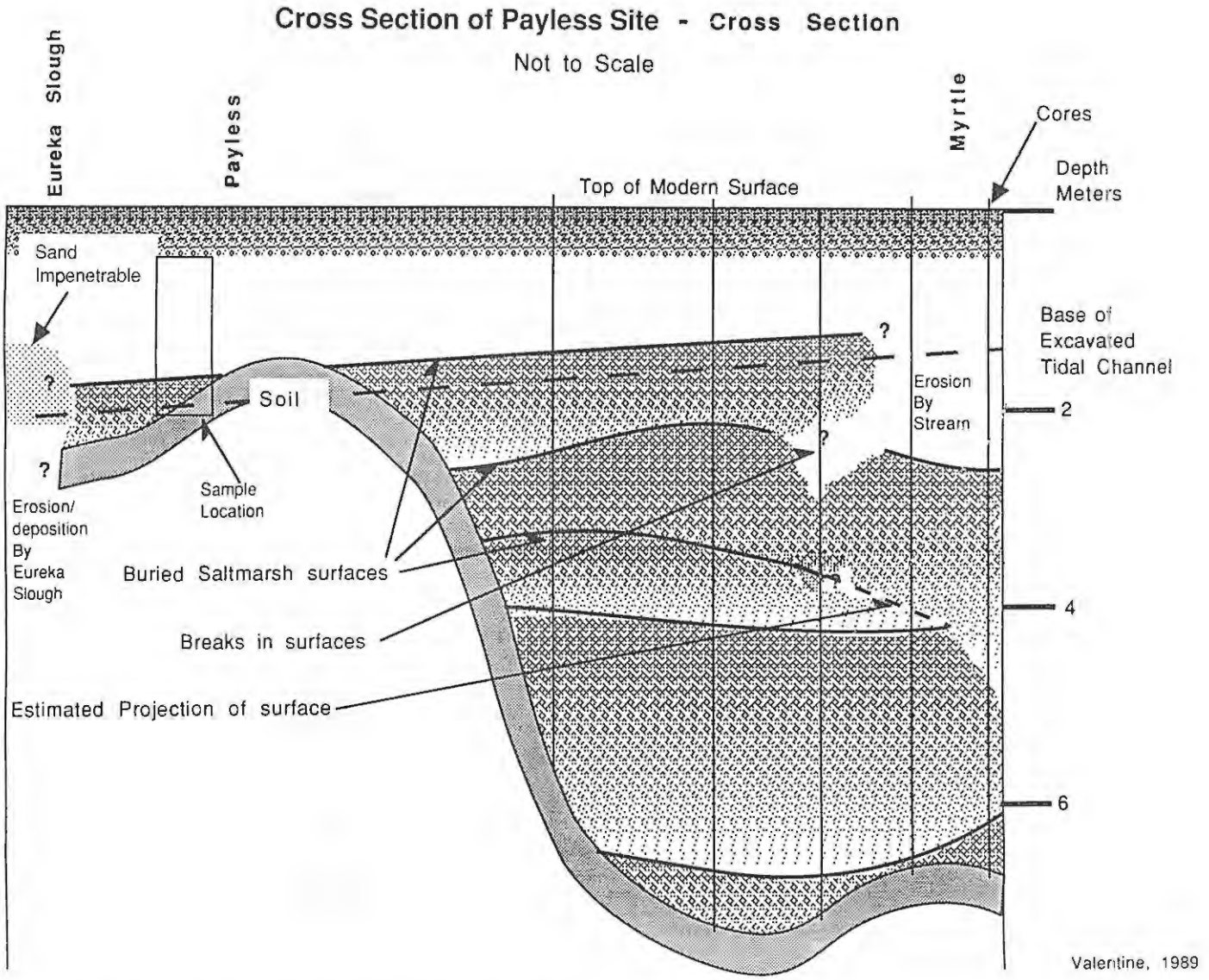

Figure 3. Payless location. The soil represents a ridge which has been buried with saltmarsh sediments by eustatic sea-level rise, and the active subsidence of the freshwater cyncline.

an indicator of subsidence events, so sediment analyses will not be useful in the distinguishing events in areas where no buried marsh surfaces are found or where a surface is thought to be missing from a sequence. Sedimentology can be useful in the reconstruction of paleoenvironments for areas of the bay.

Cooperating Organizations

California State University system
Cities of Eureka and Arcata
Humboldt County
Humboldt State University Marine Science Laboratory
National Science Foundation
U.S. Fish and Wildlife Service

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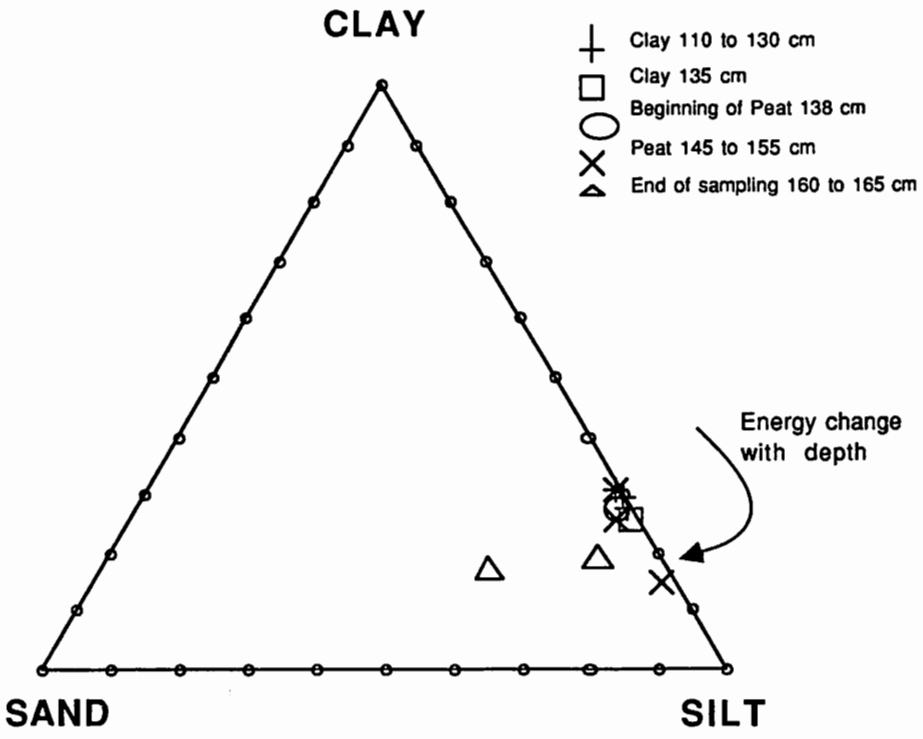

Figure 4. Size analysis data from Eureka Slough Site 2. The samples from the layer above and below the peat layer plot in the same area. There is an overall change in the depositional environment as shown by the triangles plotting in a different area.

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Aquaculture

Determination of Optimum Dietary Protein, Lipid, and Carbohydrate Levels of Hatchery-Produced Juvenile Sturgeon

University of California, Davis
R/A-67
Project Initiated: October 1, 1986
Project Completed: September 30, 1989

Silas S. O. Hung

The overall objective of the project was to acquire enough information on the nutrition of hatchery sturgeon for the development of a cost-effective commercial sturgeon feed. Specific objectives were to determine (1) the quality of different dietary proteins, lipids, and carbohydrates and (2) the optimal level of the best proteins, lipids, and carbohydrate for growth of sturgeon. An additional objective and modification of the two original objectives had been incorporated into the project in the second and third years. The additional objective was to determine the optimal feeding rates of different sizes of sturgeon kept at different water temperatures. The two original objectives were modified to determine the nonessentiality of soy-lecithin and the choline requirement of juvenile white sturgeon. We needed information obtained from these studies in order to determine the quality of different dietary lipids and the optimal level of the best dietary lipid.

An experiment was conducted to determine the dietary protein requirement of juvenile white sturgeon (Moore et al., 1988). In this experiment, juvenile white sturgeon were fed one of eight purified diets, each of which contained from 20.0% to 52.7% crude protein. Protein of these diets was supplied with graded amounts of the control protein (vitamin-free casein:wheat gluten:spray-dried egg white = 62:30:8). Percentages of 8-week growth (body weight increase, %BWI), feed efficiency (%FE), protein deposited (%PD), and energy retained (%ER) showed a high degree of agreement and were equally sensitive to the different levels of dietary protein. They were significantly ($P < 0.05$) affected by the dietary protein levels. Protein requirement of juvenile white sturgeon on an as-fed basis was estimated from the %BWI using second-order polynomial regression analysis. The regression analysis showed that the minimum range of protein requirement was between 36.5% and 40.5%. Based on these results, a 40% crude protein portion on an as-fed basis was recommended for juvenile white sturgeon feeds.

A second growth trial was conducted to determine the performance of white sturgeon fed purified diets with different proteins (Stuart and Hung, 1989). These diets were isoenergetic and isonitrogenous, and each contained one of eight different proteins as the sole protein source. Eight-week %BWI, %FE, %PD, and %ER of sturgeon were significantly ($P < 0.05$) affected by the different dietary proteins. A high degree of agreement also existed between these measurements as observed in the previous study (Moore et al., 1988). Among the four measurements, %ER appeared to be the most sensitive index because it gave the greatest degree of separation between the different proteins. The sensitivity of %ER was higher than that of %PD because the differences in body lipid were greater than the differences in body protein in sturgeon fed the different proteins. Based on the %ER, their quality was control = casein > defatted shrimp meal > defatted herring meal > soybean concentrate > egg white > gelatin > defatted zein meal.

Another growth study (Hung and Lutes, 1988) was conducted using a 2 x 2 factorial design (0% or 8% refined-soy lecithin and 0% or 0.8% choline chloride supplements) to determine the essential requirement of these two supplements in juvenile white sturgeon. Eight-week %BWI and %FE showed very similar patterns. They were significantly ($P < 0.05$) affected by each of the two supplements, and the interaction was also significant. The lower growth and feed efficiency of fish fed the diet without refined-soy lecithin and choline compared with those of fish fed diets with either or both supplements suggest these two supplements may be required by sturgeon for good growth. There was, however, little or no additive growth-promoting effect when both supplements were given. This suggests that only one of these supplements is required. Choline, a moiety common to both choline chloride and phosphatidylcholine, was present in the refined-soy lecithin. This suggested that the growth-promoting effect of refined-soy lecithin was mostly resulted from the choline in phosphatidylcholine, rather than from the refined-soy lecithin itself.

In a follow-up experiment, for 8 weeks, sturgeon were fed one of the eight diets supplemented with 0% or 8% refined-soy lecithin and 0%, 0.2%, 0.4%, or 0.8% choline chloride (Hung, 1989). Eight-week %BWI, %FE, and total plasma lipid levels had very similar patterns. They were significantly ($P < 0.05$) affected by each of the two supplements, and the interactions were also significant for the measurements. Sturgeon fed the diet without both supplements had an extensive, diffused fat vacuolation and fatty cyst formation in their liver. In the absence of refined-soy lecithin supplement, the growth, feed efficiency, and total plasma lipid levels were affected significantly ($P < 0.05$) by the choline chloride supplements. Results in this experiment suggested that there is no requirement for refined-soy lecithin, but a 0.17%–0.32% choline/kg diet (30–60 mg/kg body weight/day) is required by juvenile

white sturgeon for good growth.

A recently completed experiment showed that juvenile white sturgeon have widely different abilities to utilize carbohydrates (Hung et al., 1989a). In this experiment, sturgeon were fed isonitrogenous diets containing 27.2% of one of the eight carbohydrates. The eight carbohydrates were glucose, fructose, maltose, sucrose, lactose, dextrin, raw cornstarch, or cellulose. Eight-week %BWI, %FE, %PE, and %ER were significantly ($P < 0.05$) affected by the different dietary carbohydrates. Again, %ER appeared to be the most sensitive index to assess the ability of sturgeon to utilize different dietary carbohydrates. The greater sensitivity of this measurement also resulted from the greater differences in the body lipid of sturgeon fed the different carbohydrates. Based on the %ER, the ability of sturgeon to utilize different dietary carbohydrates in decreasing order was glucose = maltose > raw cornstarch = dextrin = sucrose > lactose = fructose = cellulose.

A feeding-rate study showed that 8-week %BWI and feed-gain ratio were significantly ($P < 0.05$) affected by the different feeding rates (Hung and Lutes, 1987). The %BWI increase showed an asymptotic curve similar to those growth curves derived from the nutrient requirement studies of mammals and fishes. The optimal feeding rate based on the %BWI was 2.0% BW/day. A follow-up feeding-rate study (Hung et al., 1989b) using a Latin-square design showed that the optimal feeding rate of sturgeon subyearlings (0.25 kg) at 18°C was between 1.5% and 2.0% BW/day.

In our study, juvenile white sturgeon did not require cholesterol or lecithin for growth, but 0.17%–0.32% of choline and 40% of crude protein were required by juvenile white sturgeon for good growth. A protein mixture (casein:wheat gluten:egg white = 62:30:8) and casein were the best proteins, whereas glucose and maltose were the best carbohydrates. The optimal feeding rates for white sturgeon weighing 15–150 g was 2.0% of BW/day at 20°C, whereas for sturgeon weighing 250–500 g, it was 1.5% to 2.0% BW/day at 18°C.

Cooperating Organizations

Department of Animal Science, University of California, Davis
Institute of Aquaculture Research, The Agricultural Research Council of Norway Sunndalsora, Norway.
Life Sciences, Bethlehem University, West Bank
The Fishery, Galt, California

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Endocrine Control of Molting and Reproduction in Decapod Crustacea

Bodega Marine Laboratory
R/A-68
Project Initiated: October 1, 1987
Project Completed: September 30, 1990

Ernest S. Chang

Lobster Molt-Inhibiting Hormone

Crustacean growth is limited by the periodic shedding of the confining rigid exoskeleton. Crustaceans must escape from the confines of their old, smaller cuticle before expansion of the soft, new one. Tissue growth then occurs to fill this larger exoskeleton. The control of molting is mediated by the steroid molting hormone 20-hydroxyecdysone. The release and synthesis of 20-hydroxyecdysone by the molting gland (Y-organ) are controlled by the neurohormone molt-inhibiting hormone (MIH). The characterization of the chemistry and physiology of MIH has been a major thrust of our research program.

We have recently completed the determination of the primary sequence of purified lobster MIH. It is a hydrophobic peptide of 71 residues that was isolated from extracts of lobster sinus glands (sinus glands are storage organs for neurosecretory hormones located in the eyestalk). MIH prolonged intermolt periods and lowered levels of ecdysteroid in juvenile lobsters.

Several thousand sinus glands were dissected from freshly excised eyestalks from intermolt lobsters (*Homarus americanus*) grown in our own culture facility (Chang and Conklin, 1983; Conklin and Chang, 1983). The glands were homogenized in 0.1 *N* HCl and heated for 5.0 min at 80°C. The extracts were purified using high-performance liquid chromatography (HPLC). A single active peak was isolated. This peptide was initially reduced and carboxymethylated to *facilitate* subsequent sequencing studies.

The primary amino acid structure deduced from Edman degradation of pyroglutamase-treated protein and peptide fragments is shown in Figure 1. This structure was assembled as follows: First, N-terminal sequencing was performed by using either spinning cup (Beckman model 890M) or gas-phase (Applied Biosystems 470A) instruments. Studies on about 20 μg (2.4 nmol) of purified, des-pyroglutamate[1]-MIH provided an initial 30 of 36 residues. The blocked N-terminal pyroglutamate was determined by (1) the inability to sequence MIH without previous treatment with pyroglutamase and (2) the shift in elution time of MIH after treatment with pyroglutamase.

Some sequence data were obtained from purified fragments from cleavages with endoproteinases (Lys-C, Glu-C, Arg-C, Asp-N). These enzymes cleave peptides at specific locations and were necessary to produce several overlapping fragments of the parent MIH molecule to allow successful sequencing. Endoproteinase Lys-C was used to cut the polypeptide on the C-terminal side of the six lysine residues predicted from the amino acid composition. Five well-resolved major peptide fragments, L1–L5, were obtained (Figure 2) and sequencing allowed confirmation of residues 9–17 (L3). The L4 peptide (residues 19–32) corrected a deletion in the original sequence and confirmed the cysteine at residue 23. Peptide L5 provided a hydrophobic C-terminal fragment, from which 19 of the 21 residues 33–53 could be

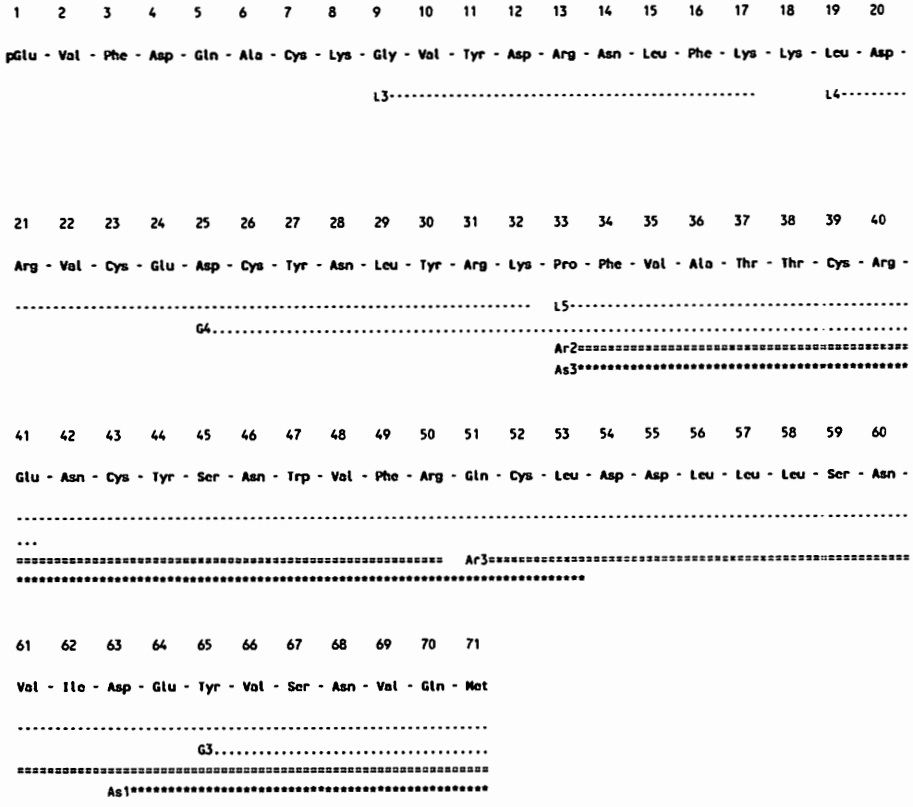

Figure 1. Primary amino acid sequence of *Homarus americanus* molt-inhibiting hormone deduced from peptide fragments. Abbreviations: L3-L5, peptides produced by cleavage with endoproteinase Lys-C; G3, G4, produced by cleavage with endoproteinase Glu-C; Ar2, Ar3, produced by cleavage of L5 by endoproteinase Arg-C; As1, As3, produced by cleavage of L5 by endoproteinase Asp-N.

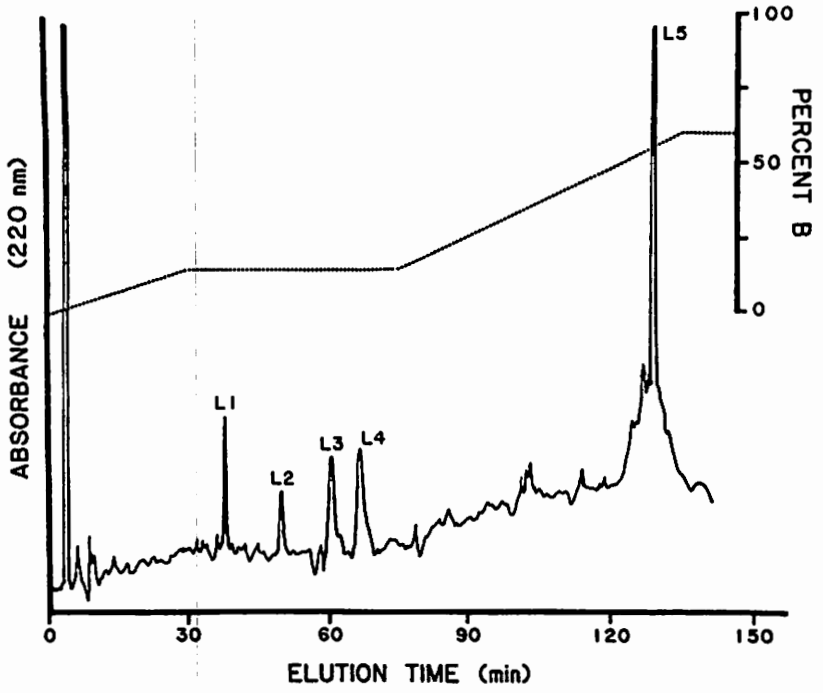

Figure 2. Preparative high-performance liquid chromatography of endoproteinase Lys-C peptides from lobster molt-inhibiting hormone. Cleavage products from approximately 75 sinus gland equivalents were injected. Solvent A was 0.1% trifluoroacetic acid (TFA) in water, solvent B was 0.1% TFA in 80% acetonitrile. Flow rate was 1.0 ml/min. The gradient was linear from 0% to 15% B over 30 min, isocratic at 15% B for 45 min, and then linear from 15% to 60% B over 60 min (broken line). The eluent was monitored at 220 nm at 0.1 absorbance units full scale (AUFS) (solid line).

assigned.

The remainder of peptide L5 was obtained with subsequent endoproteinase cleavages. It was cleaved with endoproteinase Arg-C because amino acid analysis predicted two arginine residues in L5. Ar3 was the most informative, extending the unambiguous sequence to residue 68.

L5 was also cleaved separately with endoproteinase Asp-N, because amino acid analysis predicted at least three aspartate residues (two were contiguous). The resulting three fragments (As1–As3) provided the rest of the sequence data.

The fragments produced by cleavage with endoproteinase Glu-C were used to confirm linkages. More than eight major fragments, G0–G7, could be resolved and collected for Edman degradation.

Because amino acid analysis had revealed a single methionine (not present, however, in the first 36 residues), we performed a cyanogen bromide cleavage of native MIH. After the reaction, HPLC did not show any change in the elution time of the substrate peptide. To confirm that this sole methionine residue was in fact the C-terminus, we digested native MIH with carboxypeptidase Y. The results confirmed positions 66–71.

Initial attempts with mass spectrometry to determine if the C-terminus was blocked were unsuccessful. Thus, the HPLC retention time of native As1, produced by endoproteinase Asp-N cleavage of L5, was compared with the retention times of synthesized As1 with either the Met acid or amide at the C-terminus. The results indicated a retention time consistent with an unblocked Met acid at the C-terminus. MIH from *H. americanus* thus has a calculated molecular weight of 8483, assuming that the six cysteines have formed three disulfide bonds.

This is the first reported amino acid sequence of a crustacean MIH. This peptide also has significant crustacean hyperglycemic hormone (CHH) activity. In order to assay for CHH activity, various peaks obtained on HPLC were injected into juvenile lobsters. Blood was removed before and 90 min after each injection. The hemolymph was extracted with ethanol, and the supernatant was added to a glucose oxidase color reagent (Sigma). Levels of glucose were determined by using spectrophotometry. As seen in Table 1, on a per sinus gland equivalent basis, MIH (peak 110) and peak 106 have the most CHH activity. Two other hydrophobic peaks (peak 90 and peak 102) also had significant CHH activity.

Table 1. Hyperglycemic Hormone Activity of *Homarus americanus* Sinus Gland Peptides Assayed in Juvenile Lobsters

Peak	Percent Change (± S.D.)	n
39–40	40 ± 7	5
46	38 ± 28	5
54	39 ± 49	5
61	0 ± 43	6
63	43 ± 26	5
67	57 ± 31	4
74	28 ± 48	6
85	25 ± 25	7
88	9 ± 24	7
90	200 ± 102*	6
93	23 ± 37	7
102	244 ± 88*	6
106	593 ± 118*	6
110	710 ± 187*	6
SG	435 ± 271*	10
NSG	30 ± 39	28

Lobsters were injected with purified sinus gland pepties obtained from high performance liquid chromatography. Controls were injected with non–sinus gland eyestalk neural tissue (NSG) or whole sinus glands (SG). Values with an asterisk were significantly different from NSG controls ($P<.001$).

The amino acid sequence of our MIH shows striking similarity to CHH

isolated from the shore crab *Carcinus maenas* (Kegel et al., 1989). CHH has 72 amino acid residues and a molecular weight of 8524. Sequence identity between the two peptides is approximately 61%. Thus, MIH appears to be a second member of a novel family of neurohormones. Other likely members are MIH from the crab *C. maenas* (Webster and Keller, 1986) and from the crayfish *Procambarus bouvieri* (Huberman and Aguilar, 1989) and CHH from a number of diverse crustaceans (Keller, 1981; Newcomb, 1983; Martin et al., 1984; Van Wormhoudt et al., 1984; Kallen et al., 1986; Huberman and Aguilar, 1986, 1988; Tensen et al., 1989; Soyez et al., 1990). The study of this new family of important peptide hormones will be of interest for comparative endocrinology and for the more efficient culture of crustaceans.

In addition, these data are significant because they have provided the necessary information to construct a DNA probe to begin molecular characterization of the gene that codes for MIH. From both the known N- and C-terminal amino acid sequences, we constructed several 17-mer oligonucleotide DNA probes (Wahl and Berger, 1987). We used these probes to screen a genomic DNA library of *H. americanus*. This library was constructed in the lambda virus vector EMBL-3 and plated with the *Escherichia coli* host CES200 (Frischauf, 1987).

We screened approximately 500,000 clones and isolated several that bind the probes (indicating that these clones may contain the MIH gene). These positive clones are being isolated and subcloned. They are being rescreened to verify binding of the probes.

Ecdysteroid Metabolism and Role in Molting and Reproduction

We have continued our projects dealing with the metabolism, excretion, and mode of action of ecdysteroids (a family of arthropod molting hormones). These include (1) characterization of ecdysteroids present in the blood of vitellogenic female crabs and in crab embryos; (2) determination of the concentration of ecdysteroids present in the blood of shrimp during the course of the molt cycle; (3) determination of the concentrations of ecdysteroid present during development of shrimp (*Sicyonia ingentis*) embryos; and during this last year, (4) characterization of ecdysteroid metabolites present during the molt and reproductive cycles of lobsters.

In order to obtain baseline information on the concentrations of ecdysteroids during these cycles, levels of ecdysteroid in hemolymph during the molt cycle of lobster were measured using radioimmunoassay (RIA) (Chang and O'Connor, 1979). Individual animals showed small, transitory increases in the levels of ecdysteroids that increased in magnitude with the onset of premolt

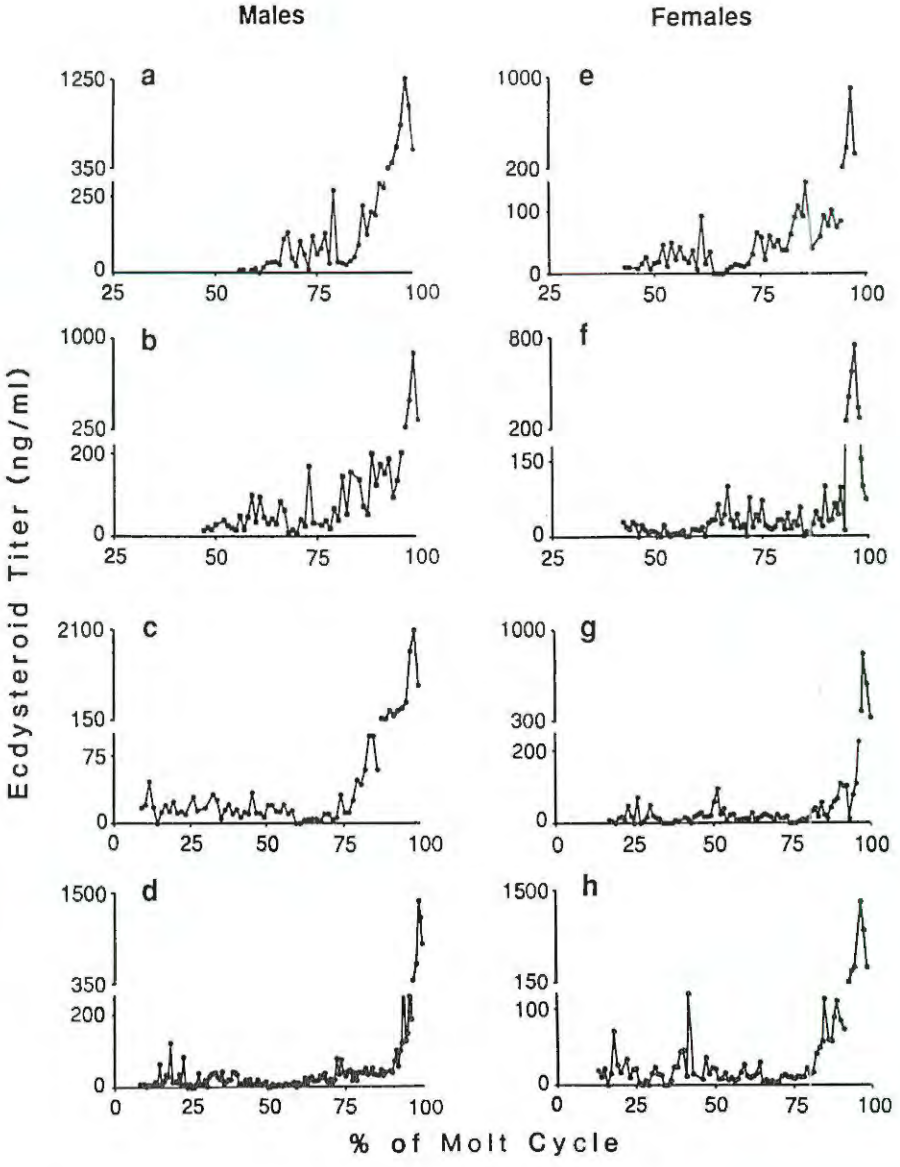

Figure 3. Titers (ng/ml) of ecdysteroid in the hemolymph of individual adult male (a–d) and female (e–h) lobsters. Every two datum points delineate 1 week, and all samples are plotted as a percentage of the entire molt cycle of each lobster. Stage C_4 extended from 10–75%, D_0 from 75–95%, D_1 from 95–98%, and D_2–D_3 from 98–100% of the molt cycle duration, respectively. Ecdysis occurred at 0% and 100% of each molt cycle. Axes are adjusted to illustrate the qualitative similarities in the hormone profiles.

and culminated in a large premolt peak at morphological stages D_2^2 to D_3^1. Male lobsters had significant postmolt peaks and late premolt levels that remained high until ecdysis. Females had no postmolt peaks, and late premolt titers reached basal levels before ecdysis.

Levels of ecdysteroid in four males (a–d) and four females (e–h) are shown in Figure 3. Titers were variable over most of the molt cycle for both sexes. For each lobster, intermolt (stage C_4) concentrations ranged from 0 to 100 ng/ml. Small, brief peaks of ecdysteroids in hemolymph were often clustered and surrounded by periods of lower basal levels. In late C_4, both the number and height of successive peaks increased with the onset of apolysis in early premolt (stage D_0; 75–95% of the molt cycle). Late-stage C_4–D_0 males and females had peaks of 200 ng/ml or less. A slight decrease in titer signified the onset of stage D_1^1 (at 95% of the molt cycle), which is delineated by a ruffled appearance on the pleopod epidermal border (Aiken, 1973). Levels of ecdysteroid then increased dramatically to final premolt peak titers of 800–2500 ng/ml. Peak levels were not significantly different between the sexes; they occurred near the transition from stage D_2^2 to D_3^1 (98–99% of the molt cycle duration).

Levels of ecdysteroid were also carefully examined through very late premolt (stages D_3^1 to D_3^{4-5}) and postmolt (stages A_1 C_3). Figure 4 summarizes these data for males and females over the molt cycle. Males had a significantly higher postmolt peak than females that occurred in stages B to C_3 (297 ± 90 ng/ml vs. 56 ± 16 ng/ml). From early intermolt (C_4 through the final peak (D_2^2 to D_3^1), levels of ecdysteroid in hemolymph were equivalent for both sexes. Males maintained significantly higher levels through late premolt (D_3^1 to D_3^{4-5}). On the day before ecdysis, mean concentrations were 646 and 148 ng/ml for males and females, respectively. Levels had dropped dramatically for both sexes and were not significantly different during the first day of postmolt (stages A_1–A_2).

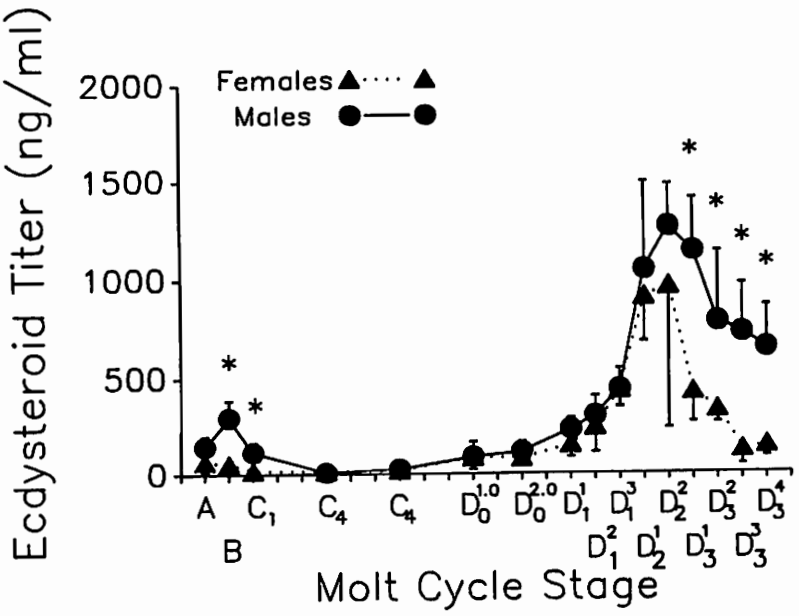

Figure 4. Titers (ng/ml) of ecdysteroid in hemolymph during the molt cycle of female (triangles) and male (solid circles) lobsters at different stages of the molt cycle. Each point represents the mean of three to seven determinations. Vertical bars indicate one standard deviation from the mean. Stage determinations are those of Aiken (1973) except that our $D_1^2 = D_1''$, $D_1^3 = D_1'''$, and $D_2^2 = D_2''$. The D_3 substages are those of Cheng and Chang (unpublished observations). Asterisks indicate significant differences at $P<.05$. Mean values at each molt stage have been connected with lines to illustrate the loss of the detailed alterations in titer at premolt, as has been done in numerous other publications.

At least seven different ecdysteroid metabolites were identified by HPLC-RIA analyses. High polarity products were the most abundant metabolites in virtually every molt stage. Levels of high polarity products were significantly higher in males than in females during late postmolt–early intermolt and in late premolt. Levels of 20-hydroxyecdysone were equivalent in both sexes and correlated with the morphological changes associated with premolt. Evidence was also obtained for the presence of ecdysone, ponasterone A, and other as yet unidentified metabolites. The pattern of ecdysteroid metabolites in the hemolymph supports other data that indicate 20-hydroxyecdysone as the major molting hormone. Metabolism of 20-hydroxyecdysone is primarily toward more polar compounds, including conjugates.

Elimination of Ecdysteroid

Patterns of ecdysteroid excretion were also followed during the molt cycle of adult male and female lobsters (Figure 5). As shown by RIA, urine was the major route of elimination, accounting for 96% or more of ecdysteroid excreted during all molt stages. The other identified route of elimination of ecdysteroid from the hemolymph was the feces, which accounted for the remaining 4%. High polarity metabolites, including 20,26-dihydroxyecdysone and 20-hydroxyecdysonoic acid, were the major types of ecdysteroids found in the urine. Other urinary ecdysteroid components included 20-hydroxyecdysone, ecdysone, and ponasterone A. The fecal ecdysteroids were mostly high polarity products and apolar metabolites.

We also conducted experiments to determine the fate of dietary ecdysteroids. By means of intubation, [^{3}H]-ecdysone was placed directly into the cardiac stomach of lobsters. The gut pathway formed an apolar conjugate of [^{3}H]-ecdysone that was found exclusively in the

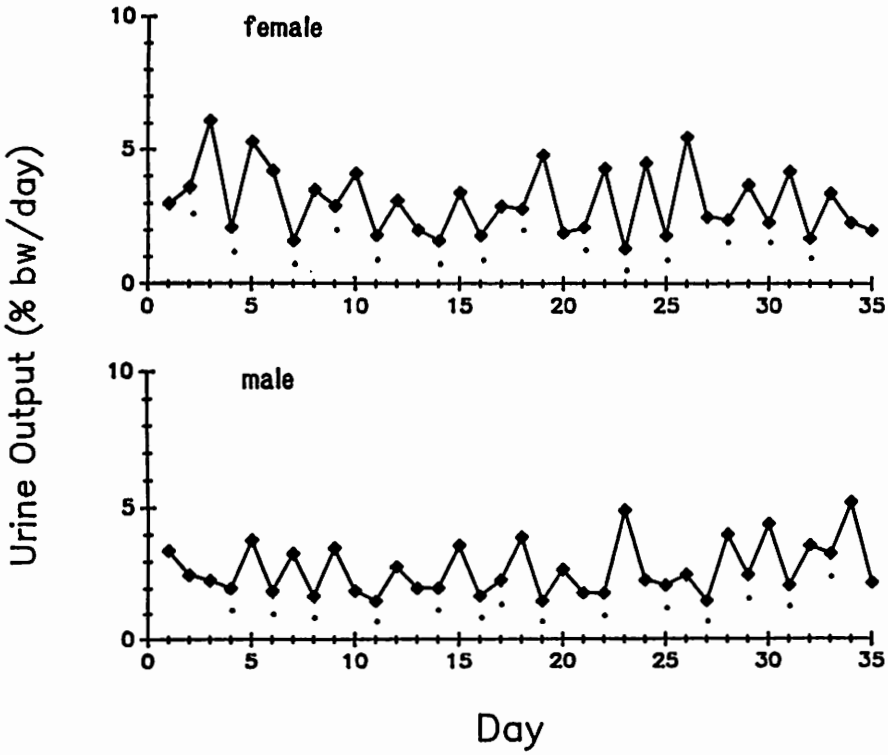

Figure 5. Urinary output from typical cannulations of female (top) and male (bottom) lobsters in mid-intermolt stage (C_4). Urine, reported as percentage of body weight, was collected daily for 35 days. Dots below data points indicate feeding days.

feces. Lobsters are therefore capable of excreting ingested ecdysteroids without absorption into the hemolymph.

Shrimp Ovarian-Inhibiting Hormone

The initial observations that provided a basis for the existence of an ovarian-inhibiting hormone (OIH) were made by Panouse (1943). He observed that, depending on the molt stage, removal of the eyestalks from the shrimp *Palaemon serratus* resulted in accelerated ovarian development and spawning. We have established a useful bioassay for the subsequent purification and characterization of the peptide OIH in *S. ingentis*. This penaeid species is ideal for the assay of OIH because it undergoes several cycles of reproduction without intervening molt cycles in the summer months.

We observed that sinus gland extracts from summer (reproductively active) shrimp are not active in inhibiting vitellogenesis. Extracts from glands obtained from winter (reproductively quiescent) shrimp are active in our OIH assay. We have dissected several hundred sinus glands from winter shrimp and have isolated a number of peptides by using HPLC. Currently, each of these shrimp sinus gland peptides is being assayed for OIH activity.

Methyl Farnesoate Binding Proteins

On the basis of the similarities between crustaceans and insects in terms of the endocrine regulation of molting, it was hypothesized that an analogue to the insect juvenile hormone may be present in crustaceans. Recent work indicates that a sesquiterpenoid other than juvenile hormone may be the modulator of crustacean development. The related compound, methyl farnesoate (MF), was isolated from the hemolymph of the crab *Libinia emarginata* (Laufer et al., 1987).

Further evidence for an endocrine role of MF in crustaceans was the demonstration of a binding protein in hemolymph similar to that found in insects (for review, see Goodman and Chang, 1985). We provided evidence for such an MF-binding protein in both shrimp (*S. ingentis*) and lobsters (*H. americanus*) by using the unlabeled and tritium-labeled hormones farnesyl diazomethyl ketone and (2E,6E)-methyl farnesoate (synthesized by our colleagues Ujvary and Prestwich, 1990).

Using these hormone analogues, we showed specific binding in the hemolymph of both shrimp and lobsters. In lobsters, the radiolabeled photoaffinity analogue forms a covalent bond with a binding protein of molecular weight 42,000 and is inhibited by 100-fold unlabeled hormone.

Although a definitive hormonal role for MF has not been established, these data strongly suggest that a specific binding protein may prevent rapid degradation of MF and may facilitate its cellular action. The role of the crustacean MF-binding protein may be analogous to that of the insect juvenile hormone-binding protein. We are currently investigating the reproductive and morphogenetic roles of the MF-binding protein in a number of different crustacean species.

Cooperating Organizations

Aquaculture Production Technology, Ltd., Jerusalem, Israel
Harvard Medical School, Boston, Massachusetts
Hebrew University of Jerusalem. Jerusalem, Israel
National Taiwan University, Taipei, Taiwan
State Lobster Hatchery and Research Station, Vineyard Haven, Massachusetts
State University of New York, Stony Brook, New York
Illinois State University, Normal, Illinois

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Intestinal Nutrient Uptake and Hormone Treatment in Fish

University of California, Los Angeles
R/A-71
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Jared M. Diamond, Nathan L. Collie, and Randal K. Buddington

The overall objective of this project was to aid in developing improved methods for enhancing fish growth by studying the effects of growth-promoting hormones on intestinal absorption. Rapid growth promoted by hormones shortens the length of the fish-culture production cycle, reducing holding time and expensive feed costs. However, the use of two well-known growth enhancers, growth hormone (GH) and the thyroid hormone triiodothyronine (T3), has been limited by problems concerning administration route, variable results, and side effects. Two examples illustrate these problems. First, adding T3 to the diet would be a practical treatment method if the resulting growth rates were not so variable (Donaldson et al., 1979). A major goal of this study was to evaluate how intestinal T3 uptake contributes to this variability. Second, with the recent success in producing transgenic fish that express GH at high levels, a practical GH treatment method appears forthcoming. Yet, how dietary requirements change during GH or T3 treatment is poorly understood. Hence, our aim was to understand how the intestine adapts to growth stimulation to aid in the design of diets that match nutrient composition to intestinal absorptive capacity.

From 14 fish species studied in past Sea Grant projects, we had developed an extensive background for our current studies in fish intestinal nutrient absorption. Our previous results can be summarized as follows:

1. Our *in vitro* method of measuring nutrient uptake does yield rates that are comparable to those measured *in vivo* and that reflect intestinal adaptation under a variety of conditions. We also have modified this technique to permit measurements of thyroid hormone uptake for use in this study.

2. Herbivorous fishes (common carp, grass carp, and tilapia) are genetically capable of higher carbohydrate absorption than are carnivorous species (striped bass and trout).

3. Both herbivorous and omnivorous (catfish and sturgeon) species adapt to differing dietary carbohydrate levels, but the carnivorous trout appears unable to do so. Thus, producing cheaper trout feeds by adding carbohydrates seems self-defeating. Yet, it remained unknown whether hormone-treated fish would exhibit similar limits on glucose uptake.

4. Pyloric ceca, blind-end tubes leading off the intestine in many fish species, are important sites of nutrient absorption. This observation led to speculation about whether the ceca are also major sites for thyroid hormone uptake.

5. Variations in intestinal amino acid absorption rates that occur in different developmental and environmental conditions are important because they affect the relative availability of essential and nonessential amino acids.

We chose to focus our current studies on rainbow trout because GH and T3 are proven growth-promoters in this economically important species (Donaldson et al., 1979). Furthermore, trout are a convenient model for studying salmonid growth regulation, and the results are relevant to California's anadromous salmon industry.

We began by examining how nutrient absorption varied with size and development in the absence of hormonal growth stimulation. Uptake of the amino acid L-proline was

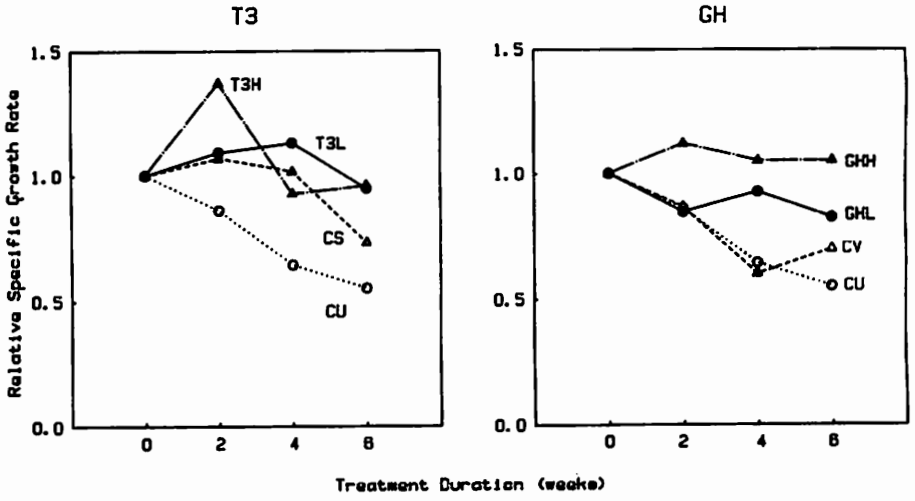

Figure 1. Effects of T3 feeding (left panel) and GH injections (right) on specific growth rate (%/day) expressed as a proportion of each group's initial rate. The relative specific growth rates of controls (CU, untreated; CS, solvent-fed; CV, vehicle-injected) decreased after 6 weeks. In contrast, the T3-fed fish (T3L, low dose; T3H, high dose) and GH-injected groups (GHL, low dose; GHH, high dose) completed the 6-week treatment period with essentially the same rate as at day 0.

measured in different gut regions and at different bathing-solution concentrations. This allowed us to determine the maximal rate of uptake (Vmax) and compare transport activity in fish over a threefold size range. In the pyloric ceca of small trout, the Vmax for proline proved to be 2.5 times greater than the Vmax of large fish. This result confirmed a pattern we have observed in several vertebrate species: the faster growth rate of younger animals is matched by a more rapid proline uptake rate.

A major portion of our study centered on the effects of T3 and GH on growth and intestinal absorptive function. We gave two doses of dietary T3 (10 and 20 ppm sprayed onto Silvercup trout pellets) and of ovine GH (0.2 and 2.0 μg/g body weight per injection given every fourth day) based on previous studies showing these doses to be effective in stimulating growth (see Donaldson et al., 1979). Three control groups consisted of fish that received an untreated diet and no injection (group CU, untreated controls); fish that did not receive an injection and were fed a diet sprayed with the solvent used to dissolve dietary T3 (group CS, solvent controls); and fish that received an injection of GH vehicle solution (group CV, vehicle controls). Fish were sampled on day 0 and after 2 and 6 weeks of treatment on a constant daily feeding ration of 2.5% body weight. At sacrifice, we measured intestinal L-proline, D-glucose, T3, and T4 uptake in the pyloric cecum, the major site for nutrient uptake in trout, we measured L proline uptake in the mid intestine also (the region beginning just caudal to the last pyloric cecum attached to the intestine and ending at the valvelike constriction demarcating the start of the posterior intestine).

Hormonal Effects on Growth

Figure 1 shows the specific growth rates (percentage growth per day) of the different groups expressed relative to their initial rate (calculated for the 2 weeks preceding day 0). Whereas the growth rates of controls generally fell throughout the study, those of hormone-treated fish either increased (T3 high dose, T3H, at 2 weeks) or remained constant (all other T3 and GH doses). Compared with untreated controls (group CU) after 6 weeks, T3 fish grew over 70% faster, and GH fish grew about 50% and 90% faster for the low- and high-dose groups, respectively. These results demonstrated that our choice of hormone treatments did stimulate growth. More importantly, it allowed us to look for intestinal adaptations associated with hormonal growth stimulation.

Effects on Intestinal Growth

Because hormone-treated fish grew faster, we expected intestinal mass and absorptive surface area of these fish to be greater than that of controls. Indeed, the linear increase in pyloric cecal gut mass (milligrams per centimeter of cecal length) with body weight explains about 60% of the variation in intestinal quantity among all the groups. However, Figure 2 shows that body weight and gut mass increased significantly in T3 and GH treatment groups only after 6 weeks of treatment. An identical pattern prevailed for the mid-gut mass as well. Thus, greater intestinal quantity contributed to higher nutrient absorptive capacity of growth-stimulated fish primarily at 6 weeks of treatment.

From three fish for each group at 6 weeks, we also preserved the whole gut in fixative and compared several different macroscopic dimensions. The pyloric ceca, mid gut, and posterior gut of T3- and GH-treated fish were greater in length and diameter compared with control fish intestinal regions. Using these dimensions, we calculated the total intestinal surface area for each fish. Fish receiving either the GH high dose (GHH) or the T3 low dose (T3L) had total surface areas that

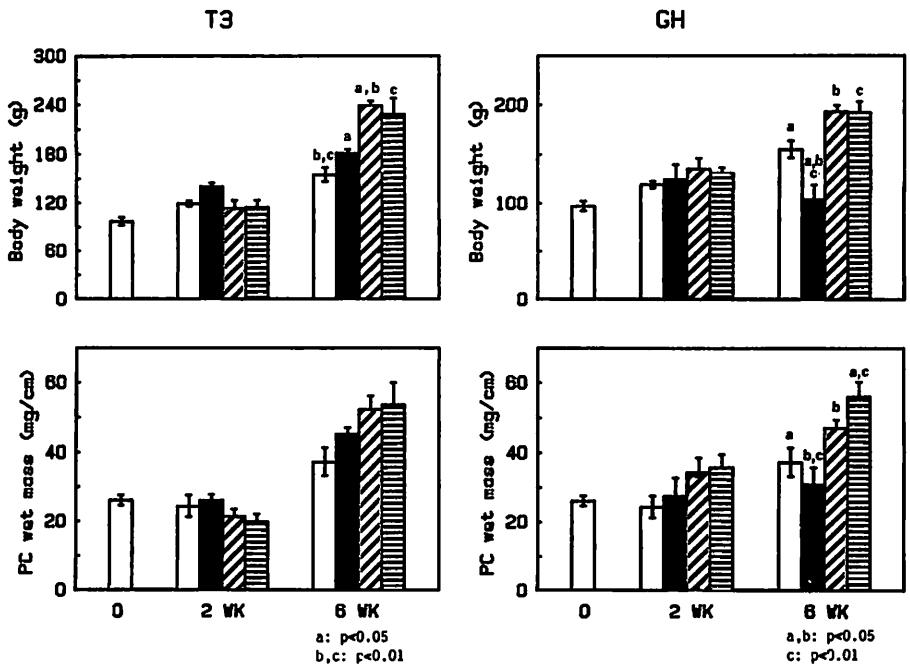

Figure 2. Changes in body weight and pyloric cecal (PC) wet mass during T3 (left two panels) and GH (right two) treatment for 6 weeks. Values for untreated controls (open bars) are repeated in the left and right sets of panels for ease of comparison. In the T3 panels, closed bars indicate solvent controls; diagonals, T3L; horizontal lines, T3H. In the GH panels, closed bars indicate vehicle controls; diagonals, GHL; horizontals, GHH. Bars with the same letters are significantly different at the probability levels indicated, determined by analysis of variance followed by the Tukey-Kramer multiple comparison test. Bar height and error symbol represent mean ±SEM.

exceeded control values by 1.4- and and 1.5-fold, respectively. But these comparisons did not take into account the larger body weights of hormone-treated fish. When we scaled gut surface area (SA) to individual body weights (assuming the geometric relationship of SA $\propto M_b^{2/3}$), the differences in surface area among the groups disappeared.

Thus, the intestine increases in mass and surface area after 6 weeks of T3 or GH treatment, but the gut does so in proportion to the rest of the body. Nevertheless, it remains unknown whether changes in intestinal morphology occur at the microscopic level. We plan to answer that question by quantifying the contribution of surface elaborations (e.g., mucosal folds) to absorptive surface area in gut histological sections from these fish.

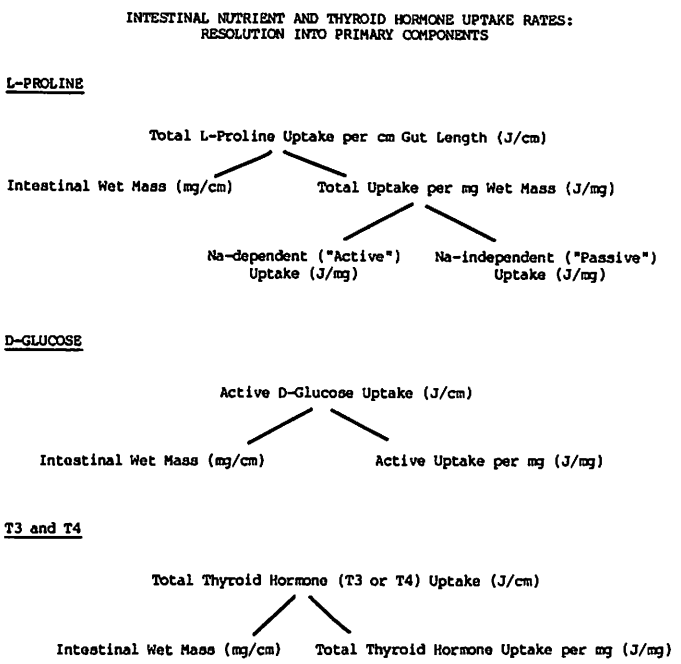

Figure 3. Diagram resolving nutrient and thyroid hormone uptake rates into primary components measured in T3-fed and GH-injected trout. The uptake rate for all solutes, J (in nM/minute for proline and glucose, or fM/minute for thyroid hormone) was normalized per centimeter of intestinal length and determined by both the tissue mass per centimeter and the uptake rate per milligram. For L-proline uptake, the total uptake rate per milligram was further resolved into Na-dependent uptake (carrier-mediated or "uphill" mechanism) and Na-independent (passive or "downhill" mechanism) components. We measured only the D-glucose uptake that is mediated by the Na/glucose cotransporter, which is the major pathway for glucose uptake at a luminal glucose concentration of 10 mM in the bath solution. T3 and T4 (1.0 nM in the bath solution) were measured as total uptake per centimeter or per milligram of intestine and not partitioned into further components.

Effects on Nutrient Uptake

What factors contribute to nutrient uptake rates? The major components are depicted schematically in Figure 3. For example, the uptake rate (J, in nM/min) for a given solute expressed per centimeter length of intestine (J/cm) depends not only on the tissue mass per centimeter but also on the transport rate per milligram of tissue (J/mg). Hence, J/cm may increase because tissue mass increases per centimeter (see Figure 2), because the intrinsic transport activity J/mg increases, or because both change.

Proline Uptake

We further resolved total L-proline uptake into Na-dependent ("active") and Na-independent ("passive") components since GH had been previously shown to alter both transport pathways in coho salmon intestine (Collie and Stevens, 1985). Proline uptake was measured at bathing-solution concentrations of 5 and 30 mM in the pyloric ceca (where many tissues may be sampled) and at 5 mM only in the mid intestine (where tissue quantity is limited).

Dietary T3. Figure 4 shows the effect of T3 feeding on the different components of L-proline uptake (measured at 5 mM) in pyloric ceca. Injection of low-dose T3 (J/cm, Figure 4, left) stimulated total uptake twice that of untreated (CU) or solvent (CS) control fish ($P < .01$). Since tissue mass per centimeter was similar in the different groups at 2 weeks of treatment, the increase was entirely due to a twofold change in J/mg (Figure 4, top right panel). Both Na-dependent and Na-independent uptake components contributed to the higher uptake rates in T3L fish. After 6 weeks of treatment, higher gut mass (T3H, $P < .01$) as well as slightly higher uptake per milligram (T3L fish) led to approximately 50% increases in J/cm of both T3-treatment groups.

In the mid intestine, low-dose T3 feeding elicited a similar doubling of L-proline uptake (J/cm) at 2 weeks as well as at 6 weeks. Again, both active and passive uptake components were stimulated in the 2-week group, whereas the higher proline uptake at 6 weeks was entirely due to increased active proline uptake. Thus, the similar pattern of uptake changes in the pyloric ceca and mid intestine of T3L fish suggests the intestine responds along its length to T3 feeding.

Tissues from T3-fed fish incubated in 30 mM L-proline generally reflected the pattern seen for uptake at 5 mM: proline uptake was increased twofold in the T3L group, with increased Na-dependent uptake primarily responsible for the higher rates. The fact that active proline uptake was increased at low and high concentrations of proline indicates that the maximal transport rate (Vmax) was greater in T3L fish, particularly at 2 weeks. This in turn suggests that an early response to T3 treatment may involve an

induction of proline transporters or a steepened Na-gradient across the luminal membrane.

GH Injections. At a bath concentration of 5 mM L-proline, both GH doses significantly stimulated uptake (J/cm) at 2 weeks by almost 75% compared with either untreated or vehicle-injected (CV) fish ($P < .05$, Figure 5). Unlike dietary T3 treatment, however, the increase was largely due to increased tissue mass. At 6 weeks, J/cm was not significantly elevated because the higher tissue mass per centimeter in GH fish was offset by lower transport activity per milligram. In addition, despite a 30% increase in tissue wet mass, GH did not enhance proline uptake in the middle intestine (J/mg values were significantly lower than controls).

At the 30-mM proline concentration, the high GH dose significantly increased J/cm at 2 weeks by 66% ($P < 0.01$). Both greater tissue mass and increased J/mg contributed to the higher uptake rate. There was a dose-related increase in Na-dependent proline uptake of 1.5- and 2.1-fold, respectively, in GHL and GHH fish. In contrast to T3-fed fish, passive proline permeability showed no increase in GH fish; in fact, Na-independent uptake was significantly reduced by the low GH dose (GHL).

We found several differences between the two hormone treatments. Low-dose T3 produced higher uptake rates (J/cm) than did either GH dose at both time points and at both proline concentrations. At the 5- and 30-mM proline concentrations, T3 stimulated uptake per milligram through active as well as passive pathways; GH increased active uptake only at the 30-mM proline concentration and reduced or left unchanged passive proline permeability. The early intestinal response to GH was an increase in tissue mass, while that for T3 was primarily increased transport activity per milligram. After 6 weeks, increased tissue mass and J/mg both contributed to higher proline uptake in T3-fed fish. For GH, the late effect on uptake was entirely due to greater intestinal quantity associated with

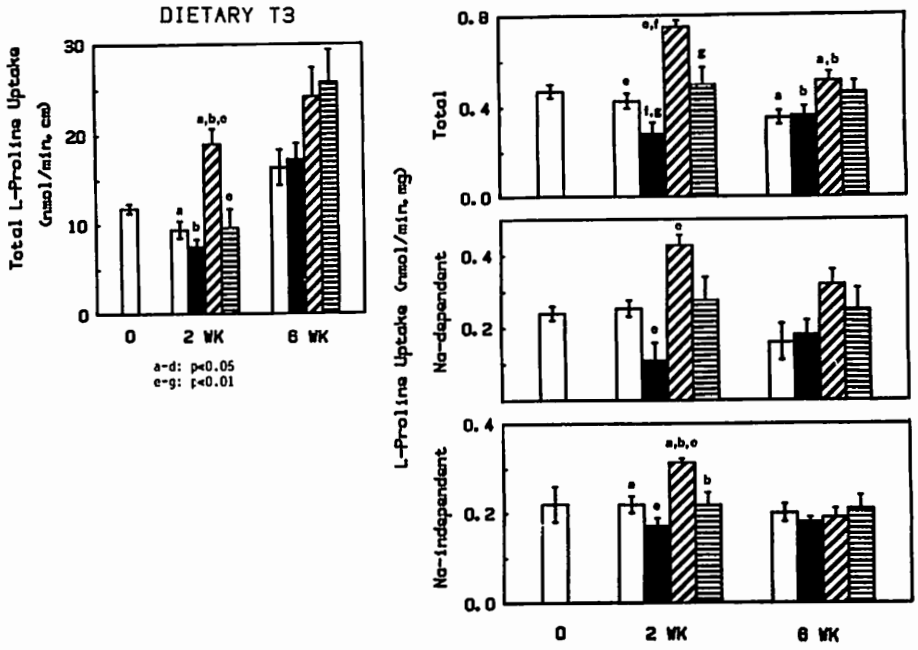

Figure 4. L-proline uptake (at 5 mM in the bathing solution) by the pyloric ceca and the effect of dietary T3 feeding. The single panel at left shows total proline uptake per centimeter of cecal length (J/cm). The total proline uptake per milligram of tissue mass (top right panel) is resolved into its two transport components, Na-dependent (middle right) and Na-independent (bottom right) uptake per mg. In the panels, open bars indicate untreated controls; closed, solvent controls; diagonals, T3L; horizontals, T3H. Statistical comparisons and representations are as described in the legend for Figure 2.

body weights that were higher than those of control fish.

Glucose Uptake

For glucose uptake, we measured the active, carrier-mediated component since it represents the primary absorptive mechanism at the 10-mM glucose concentration used in the bathing solution. This concentration results in glucose rates close to the Vmax for the glucose transporter (Buddington et al., 1987).

Dietary T3. After 2 weeks, the low-dose T3 feeding resulted in rates of active glucose uptake per milligram that were 65% and 75% higher than rates in CS and CU fish, respectively ($P < .01$, Figure 6). Neither dose significantly altered J/cm, however, since the 20% lower tissue mass of T3 fish at 2 weeks offset any increase in J/mg. By 6 weeks, T3L fish showed 2.3-fold higher J/cm values, due mainly to a twofold increase in transport activity per milligram.

GH Injections. Figure 6 shows that active glucose uptake per centimeter of GHH fish was twice that of control fish ($P < .05$), which was due almost entirely to a twofold increase in J/mg. At 6 weeks, J/cm was double that of controls for both GH doses, although the mechanisms underlying those increases were quite different for the two groups. Increased glucose uptake per milligram was largely responsible for the stimulation of J/cm in GHL fish; in the high-dose group, the primary factor was greater tissue mass per centimeter.

Thus, both hormones stimulate active glucose uptake per milligram in addition to their positive effects on intestinal growth. In contrast, previous studies concluded that the carnivorous trout, which consumes little carbohydrate in its natural diet,

was incapable of adaptive increases in intestinal glucose uptake in response to increased dietary carbohydrate levels (Buddington et al., 1987; Buddington and Hilton, 1987). The present data suggest, however, that the rate of glucose uptake is not fixed but instead increases during hormonal growth stimulation.

Thyroid Hormone Uptake

Total thyroid hormone uptake was measured using our *in vitro* intestinal sleeve preparation at 1 nM in the bathing solution, with ^{125}I-labeled T3 or T4 serving as the probe. For other measurements of amino acid uptake, we used ^{3}H-labeled polyethylene glycol (molecular weight, 4kd) to correct for fluid adhering to the everted mucosa.

T3 feeding. Trout fed T3 for 2 weeks exhibited a dose-related increase in T3 uptake per milligram of 1.8- and 2.2-fold for the low and high doses, respectively, compared to CU controls (Figure 7, left). This increase was not significant in uptake normalized per centimeter, because tissue mass in CS fish was slightly greater than T3-fed fish at 2 weeks. By 6 weeks, however, J/cm in T3L fish was twice that of controls; both higher uptake per milligram and greater tissue mass contributed to this increase.

The effects of T3 feeding on T4 uptake (Figure 8) showed a qualitatively similar pattern of change. T4 uptake (J/mg) increased progressively with the T3 dose at 2 weeks, while the lower dose produced the greatest increase in T4 uptake (J/cm and J/mg) at 6 weeks. Throughout T3 treatment, T4 uptake was generally lower than that of T3. Together, these results suggest that T3 and T4 may share the same uptake mechanism, perhaps with slightly differing affinities for the two hormones.

GH Injections. In contrast to T3 feeding, GH had little effect on T3 transport activity per milligram at 2 and 6 weeks of treatment. Hence, the twofold-higher uptake per centimeter at 2 and 6 weeks, respectively, for GHH and GHL fish compared with solvent controls was

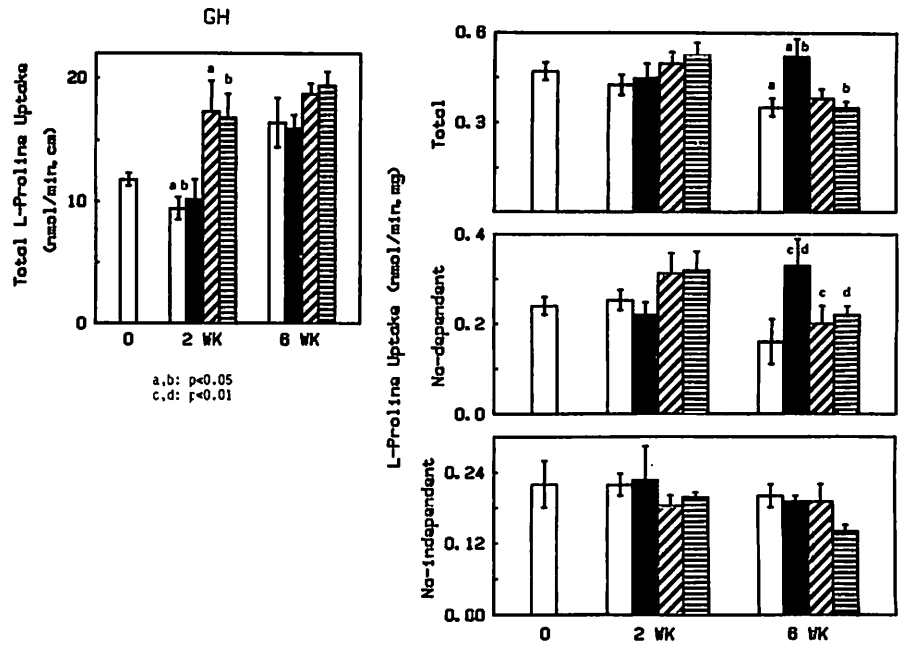

Figure 5. Effects of GH on proline uptake by the pyloric ceca. Panels are arranged as in Figure 4 to partition uptake at the 5-mM concentration of proline into its various components. Open bars indicate untreated controls; closed, vehicle-injected controls; diagonals, GHL; horizontals, GHH. Statistical symbols are as described in the legend for Figure 2.

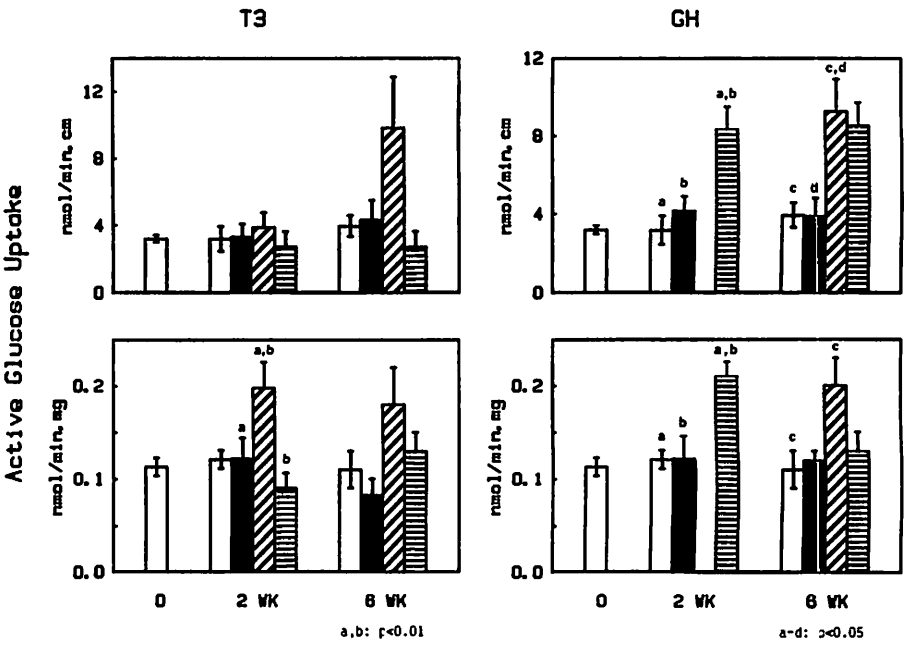

Figure 6. Hormonal stimulation of active glucose uptake expressed per centimeter (top panels) and per milligram tissue mass (bottom). Bars are coded as in Figure 2, except that no GHL bar (diagonal pattern) appears at 2 weeks because the samples were unusable owing to technical problems.

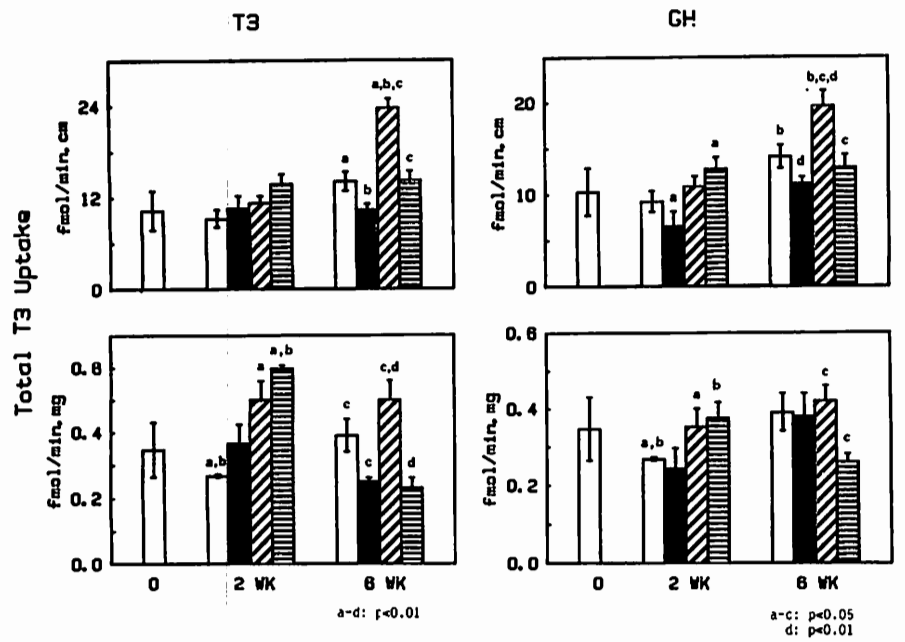

Figure 7. Total T3 uptake by pyloric ceca (J/cm, top panels; J/mg, lower panels) in T3-fed and GH-injected trout. T3 concentration in the bath solution was 1.0 nM. Bar coding and other symbols are described in the legend for Figure 2.

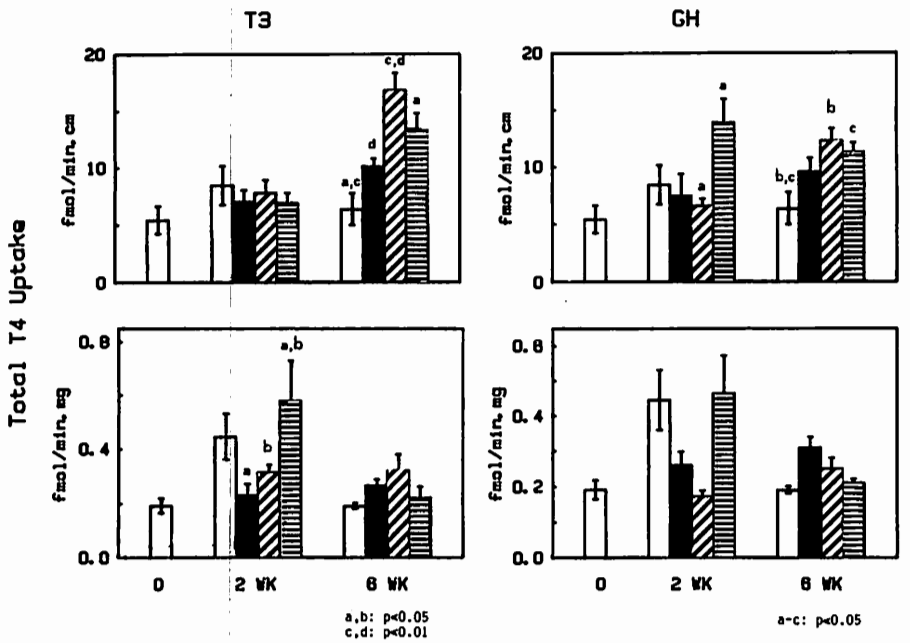

Figure 8. Thyroxine uptake (T4) during growth stimulation by T3 and GH. T4 uptake in the presence of 1.0 nM of T4 is expressed as described in Figure 7 for T3 uptake rates. Bar coding and other symbols are described in the legend for Figure 2.

caused by greater tissue mass in the GH-injected fish. The primary difference between the response of T3 and T4 uptake to GH was in the low-dose treatment group. Both at 2 and 6 weeks, the T3/T4 uptake ratio in GHL fish was significantly greater than 1.0 (1.9 ± 0.3 and 1.7 ± 0.1, respectively).

In many respects, the changes in the thyroid hormone uptake pattern in T3-fed trout is reminiscent of nutrient transporter regulation in response to dietary substrate levels. For example, high levels of carbohydrate in the diet induce greater glucose uptake per milligram, particularly in omnivorous and herbivorous species, including fish (Karasov et al., 1985). On the other hand, excessive levels of some substrates (e.g., calcium, iron, and toxic amino acids) repress the intestinal uptake rates for those solutes (Ferraris and Diamond, 1989). The thyroid hormone uptake pattern ranges between these two extremes, depending on the duration of T3 feeding. High dietary T3 first stimulated uptake at 2 weeks and then repressed uptake at 6 weeks. Hence, the great variability in past attempts to stimulate fish growth with dietary thyroid hormone feeding may be partly explained by variable hormone uptake rates during different growth phases. Our results also suggest that changes in plasma GH (e.g., during growth, development, or seawater adaptation) may influence thyroid hormone uptake by increasing intestinal mass and absorptive capacity.

Characterization of Intestinal T3 Uptake

Our measurements in T3-fed trout indicated that thyroid hormone uptake was regulated in a manner similar to carrier-mediated nutrient transport. Two primary characteristics of active nutrient uptake are its Na-dependency (i.e., when the absence of sodium in the bathing solution blocks active nutrient uptake) and saturation kinetics (i.e., the uptake vs. nutrient concentration plot is curvilinear). Hence, we examined T3 uptake for evidence of these two features. T3 kinetics were measured exclusively in the pyloric ceca, since preliminary results showed that uptake rates in that region were about threefold and fivefold higher than in the mid and posterior intestine, respectively.

Comparing T3 uptake in Na-containing and Na-free (choline)

Ringer solution, we found that sodium removal significantly inhibited T3 uptake (Figure 9). The percentage of total T3 uptake inhibited ranged from 58% at 0.1 nM to 85% at 50 nM. As for nutrient uptake, Na-independent T3 uptake was a linear function of the bathing solution T3 concentration, while total T3 (Na-dependent plus Na-independent) uptake was clearly curvilinear. Plotting the Na-dependent component vs. T3 concentration revealed a curve conforming to the Michaelis-Menten kinetics typical of active glucose and proline uptake.

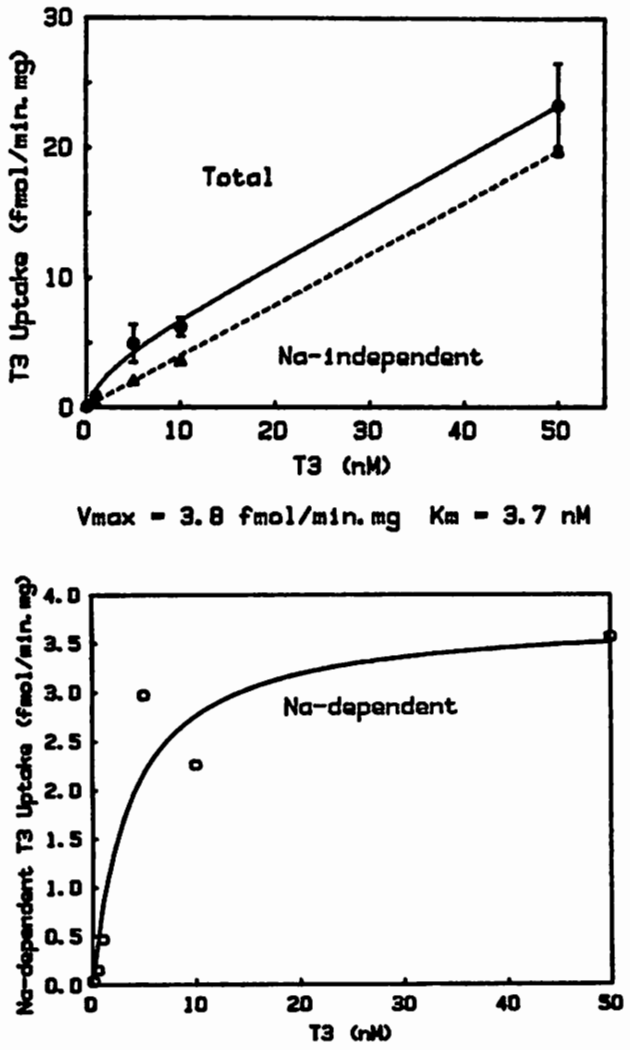

Figure 9. Mechanism and kinetics of T3 uptake by trout pyloric ceca. Top: Total T3 uptake (solid line) measured in Na-containing Ringer solution is a curvilinear function of T3 bath solution concentration in the nanomolar range. Measuring T3 uptake in Na-free solutions at different T3 concentrations yields the linear, Na-independent ("passive") T3 uptake component (dashed line). Error bars (± SEM) not shown are smaller than the symbols (circles and triangles) representing the mean uptake rates. Lower panel: The Na-dependent component was obtained by subtracting Na-independent uptake from the total T3 uptake shown above. By fitting the Na-dependent uptake to the Michaelis-Menten equation, we obtained the maximal uptake rate (Vmax) and half-maximal concentration (K_m) for carrier-mediated T3 uptake.

To our knowledge, these results provide the first evidence for a mechanism of active uptake of thyroid hormone in vertebrate intestine. But why should the gut have a transporter that facilitates T3 uptake?

Specker (1988), reviewing gut-thyroid relationships, points out that the most primitive route for thyroid hormone delivery was probably from the gut lumen into the body. Lower chordates and larval lampreys produce thyroid hormone in the gut lining (specifically, from the endostyle) and release them into the gut lumen. A mechanism facilitating thyroid hormone entry across the gut may have conferred an adaptive advantage at some stage in gut-thyroid evolution. What that advantage might have been is presently unclear, since we know little about the physiological role of thyroid hormone in these early chordate groups. Possibilities range from the enhanced retention of iodide itself to the signaling of mitogenic and metabolic actions in the intestine (Specker, 1988).

Three recent findings from studies in mammals further support an intimate functional relationship between the thyroid hormone and intestinal uptake. First, more than 50% of the body's total pool of T3 (DiStefano, 1988) resides in the gut lumen. Second, DiStefano (1988) also found that the intestinal pool freely exchanges with hormone in the circulation (i.e., enterohepatic recycling) rather than merely representing "excreted" thyroid hormone. Third, the gut absorbs free T3 and T4, rather than conjugated hormonal forms (e.g., glucuronidated T3/T4). These observations suggest that thyroid hormone uptake by the intestine may play an important role in thyroid metabolism.

Summary

A general pattern in our developmental studies of several species, including trout, is that the faster growth rate of younger animals is matched by more rapid absorption

of amino acids. This proved to be the case for proline uptake in rainbow trout differing threefold in body size.

It is known that thyroid hormone and GH play critical roles in coordinating growth, development, and salinity adaptation in salmonids. We show here that these hormones enhance intestinal L-proline and D-glucose uptake in growth-stimulated trout. The early responses to hormone treatment included changes in transport activity per milligram of intestine, particularly for T3-fed fish, as well as moderate increases in gut mass (GH fish). These changes *preceded* demonstrable increases in body growth. After 6 weeks of treatment, when size differences became readily apparent, greater intestinal mass predominated as the mechanism leading to higher nutrient uptake rates, especially in GH-injected fish.

We conclude that hormonal growth promotion results in an altered pattern of nutrient uptake. In turn, this suggests that higher growth rates might be achieved by optimizing feed quantity and composition to match absorptive capacity in hormone-treatment programs. In our study, T3- and GH-treated fish grew faster despite receiving the same ration given to untreated groups. It may be unnecessary then to give hormone-stimulated fish more food to achieve faster growth, and thus feed costs could be reduced. Furthermore, since both hormones augmented glucose uptake, cheaper feeds with higher carbohydrate content may yield adequate growth during hormone treatment without resorting to expensive, high-quality protein diets. Thus, these suggestions warrant rigorous testing in diet-controlled growth trials.

Our results provide the first evidence that T3 is taken up by an active, carrier-mediated process and that T3 feeding regulates intestinal thyroid hormone uptake. Rather than being absorbed passively, with uptake being a simple function of absorptive surface area, thyroid hormone uptake varies with hormone dose, treatment duration, and intestinal region (e.g., pyloric ceca vs. posterior intestine). T4 uptake rate is generally less than that of T3 in tissues from the same fish. Thus, the lower growth rates in T4-fed vs. T3-fed trout, and the variable growth effects of dietary thyroid hormone observed by others may result in part from the specificity and pattern of thyroid hormone uptake by the gut.

Cooperating Organizations

Bioproducts, Inc.
Calaqua
California State Department of Fish and Game
Fish Breeders
The Fishery
Hawaii Institute of Marine Biology
Institute of Marine Biochemistry, Aberdeen, Scotland
Pacific Aqua Farms
Star Milling Company
University of California, Berkeley
University of California, Davis
Whitewater Trout Company
Widman Fish Farm

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Fatal Inflammatory Bacteremia and Its Association with Summer Mortality in Pacific Oysters

University of California, Davis
R/A-74
Project Initiated: October 1, 1988
Project Completed: September 30, 1990

Ronald P. Hedrick

The overall objectives of this project are to characterize and determine the significance of the bacterium that causes Pacific oyster nocardiosis (PON) or fatal inflammatory bacteremia (FIB) in Pacific oysters (*Crassostrea gigas*) and its relationship to the phenomenon of summer mortality.

Results

Gross Pathological Changes. Affected oysters had few to no external signs of infection. Some gaped or had weak shell closure. The mantle of diseased oysters appeared normal or slightly discolored or contained raised yellow, green, or brown nodules.

Microscopic Pathological Changes. Smears made from nodules contained mostly host hemocytes and bacterial colonies. Filamentous and branched bacteria were also found within several tissues of infected *C. gigas*. The bacterium was either gram-positive or gram-variable, moderately acid-fast and PAS-positive. Bacterial aggregates appeared beaded in some instances. The bacterium was observed in almost every tissue of infected oysters from the field. However, colonies were found primarily within the gonadal follicles and the vesicular connective tissue surrounding the gut and, to a lesser extent, in the mantle, connective tissue around digestive diverticula, gills, heart, and adductor muscle. The digestive diverticula and nephridium were only rarely infected. The bacterium invoked a massive infiltration of host inflammatory cells (hemocytes) into the affected area. Hemocytes surrounding the colonies formed rosettelike arrays. Cell necrosis was observed only in very advanced cases. Small aggregates of bacteria were observed within hemocytes and have been observed diapedesing across intestinal epithelia.

Bacterial Isolation. Gram-positive bacterial colonies were detected between 3 weeks to 3 months after inoculation of brain-heart infusion (BHI) agar, BHI + 2% NaCl, BHI + 2% NaCl and 1% oyster hemolymph, Lowenstein-Jensen agar, Loeffler's egg media, Middlebrook 7H10 agar, actinomycete isolation agar, and Sabauroud dextrose agar (pH adjusted to 7.0). Colonies were isolated from the hemolymph and/or adductor muscle pustules of six Pacific oysters and from nodules of an infected heart in a seventh animal. Three of the isolates originated from oysters reared in Rocky Bay, Washington (RB1, RB13 and RB29). Two were from Oakland Bay, Washington (OB3P and OB5H). The final two isolates were cultured from the hemolymph of two infected oysters: NB4H from Nanoose Bay, Vancouver Island, and CSIHb from Scott Island, British Columbia.

Bacterial Identification. Bacteria isolated from infected oysters were filamentous, branched, and beaded. They stained gram-positive or gram-variable, acid-fast and PAS-positive. All isolates were catalase positive.

Transmission Electron Microscopy. The bacterium isolated from diseased oysters was emarginated by a trilaminar cell wall, which is typical of bacteria in the genus *Nocardia*. Pseudobranching was observed in the micrographs of the oyster isolates as compared with true branching shown by *Nocardia asteroides*.

Physiological, Chemical, and DNA Analysis. All seven isolates from diseased oysters were slow growing, had a trilaminar Lechevalier type IVA cell wall chemotype, contained nocardomycolic acids with 44 and 58 carbon numbers, contained a DNA base composition average of 68.80 mol% G + C and did not use or hydrolyze many organic and inorganic substrates.

Indirect Fluorescent Antibody Tests. Indirect fluorescent antibody analysis of hyperimmune rabbit serum to bacterial isolate RB29 or isolate NB4H showed specific affinity for all the bacterial isolates from diseased oysters; preimmune antisera did not react in any of the tests. The antisera also showed reactivity to other nocardial bacteria tested including *N. asteroides* and *Nocardia seriolae*.

Pustules induced by injection of the bacterial isolates into the adductor muscles also reacted strongly with the rabbit antisera. However, no individual bacteria could be distinguished. Smears of adductor pustules induced by injection of sterile 2% saline did not contain *Nocardia* and showed either no or background fluorescence. Pustule smears from naturally occurring lesions fluoresced brightly, as did the induced pustules. Again, no individual bacteria could be discerned.

Geographical Distribution and Seasonality. Pacific oysters from six of 12 culture sites sampled were infected with the bacterium. These locations included the three Canadian embayments, Nanoose Bay, Scott Island, and (Henry Bay) Denman Island, and the three southern Puget Sound sites, Oakland Bay, Rocky Bay, and Mud Bay. Despite earlier reports of FIB in Willapa Bay, none of the animals in our samples that were reared in Willapa Bay were infected with the bacterium.

Approximately equal numbers of males and females were examined at each location. No evidence of sex-related susceptibility to bacterial infection was observed in August

1986. However, Oakland Bay oysters sampled over 5 months in 1986 showed a trend of 2.5 times more infected males than females (32/69 males vs. 13/69 females and 24/69 immatures from a total of 226 sampled). During early to midsummer, approximately 16–22% of the Oakland Bay oysters had FIB. Infection levels increased in late August (37%) and peaked in September, when 58% of the Oakland Bay *C. gigas* harbored the gram-positive bacterium. Subsequent samples in October and November showed a decreasing prevalence of PON (19% and 31%, respectively). Unlike the geographical survey, fewer female Pacific oysters were infected than either males or sexually immature oysters.

Cohabitation Experiments. Nocardial infection was not transmissible via the water as evidenced by lack of detection of bacterial infection in the control oysters after 3 months of cohabitation with diseased *C. gigas* from Nanoose Bay (in the first study) and 6 months of cohabitation with infected Scott Island animals (in the second study). Histological examination showed that initially, 15% of the Nanoose Bay animals and 38% of the Scott Island animals had a nocardial infection. Ninety-five percent (36/38) of the Scott Island animals that died were heavily infected with *Nocardia*, whereas only 41% (9/22) of the Nanoose Bay animals that died were infected with *Nocardia*. None of the control oysters had nocardial or any other bacterial infection.

Sediment-Borne Transmission. None of the oysters incubated with Rocky Bay sediments became infected and the bacterium was not isolated from the sediments. In addition, oysters collected from Rocky Bay along with the mud samples were disease-free.

Similar results were obtained in the second experiment, in which sterile soil was inoculated with relatively low levels of the bacterium isolated from diseased Pacific oysters (~1 mg bacteria/ml soil).

Koch's Postulates. Fifty percent of the Humboldt Bay animals died within 1 month after bacterial challenge. None of the control Humboldt Bay oysters died during the experiment. All challenged oysters that died had nocardial infections and associated pathological changes. The connective tissues surrounding the gut and gonad follicles were most frequently colonized by the injected bacterium. The adductor muscle and gills were also generally colonized. Bacterial colonies were observed within the heart and surrounded by hemocytes in many animals. Oysters that died during the first 1–2 days after injection showed few to no pathological changes compared with oysters that died later in the experiment. Bacterial colonization was accompanied by an infiltration of inflammatory cells into affected areas. Acid-fast, gram-positive bacteria were reisolated from both hemolymph and adductor muscle pustules from challenged oysters only. No nocardial infections or associated pathological changes were detected in control animals.

Discussion

Geographical Distribution and Seasonality. FIB and the phenomenon of summer mortality overlap both temporally and geographically (Imai et al., 1965; Perdue, 1983; Elston et al., 1987). Of the six locations where FIB historically occurs, all have experienced summer mortality (S. Bower, Pacific Biological Station, Nanaimo, B.C., Canada, personal communication; Beattie et al., 1988). Both the bacterial disease and oyster mortality occur during the summer to early fall months when water temperatures and nutrient levels are elevated and oysters show marked gonad development (Beattie et al. 1988). These conditions may act as external stressors, which may increase an oyster's susceptibility to other adverse phenomena such as bacterial infection. Tamate et al. (1965) noted this same relationship between host, pathogen, and environment in Pacific oysters reared in Japan. These observations suggest that the gram-positive bacterium may be an opportunistic pathogen of oysters and that the bacterium and associated disease may augment mortality in certain areas. This contention supports the conclusions of Elston et al. (1987), yet is in contrast to those of early studies in Japan. Elston et al. (1987) observed, on the basis of the relatively high prevalence of infection in the southern Puget Sound embayments sampled (27–31%) and histopathological changes, and timing of the disease, that FIB can be fatal to infected oysters. In contrast, the Japanese researchers did not detect a positive correlation between the bacterial infection and mortality (Imai et al., 1965; Numachi and Oizumi, 1965; Kanno et al., 1965). Despite this, Tamate et al. (1965) concluded that multiple abscesses was a serious condition, which implies that the condition was thought to affect the host adversely.

My colleagues and I noted no trend of sex-related susceptibility in our geographical survey. However, the 69 infected oysters of 226 animals sampled from Oakland Bay (stock 388) from July to November 1986 showed that roughly 2.5 times more males were infected than females. In addition, a large proportion of infected individuals (24/69) did not have reproductive development. Thus, cumulatively, more males and sexually immature oysters appear to be affected by FIB than female oysters. These results are in agreement with the observations of Sholz et al. (1973), who observed more female oysters in embayments affected by summer mortality and deduced that mortality is more selective toward male oysters. Elston et al. (1987) also noted that more males (23%) than females (5%) were infected with the gram-positive bacterium. Reproductively immature oysters were most often infected (72%), and the authors suggested that a relationship may exist between bacterial infection and reproductive status.

Transmission Studies. The inability to transmit nocardial infection via cohabitation or with sediments indicates that other factors are involved in natural episodes. The laboratory conditions, despite fairly high temperatures (22°C), may be considered ideal, as the incoming

water was clean and well oxygenated. Oysters were not exposed to prolonged low tides and afternoon sun and were well fed daily. Transmission in the absence of these stressors may have precluded transmission of the bacterial infection in the laboratory.

Koch's Postulates. Pacific oysters injected with the bacterium isolated from diseased oysters had the same pathological changes as animals in the field that had FIB. Many of the animals injected with the bacterium died, whereas the control oysters survived, and the same bacterium was reisolated, in pure culture, from challenged oysters only. As in earlier studies both in Japan (Imai et al., 1968) and in the United States (Sinderman and Rosenfield, 1967; Elston et al., 1987), bacterial foci were primarily observed in the vesicular connective tissue surrounding the gut and within reproductive follicles. These results, in conjunction with tinctorial and morphological characteristics, fulfill Koch's postulates, proving that this bacterium is the etiological agent of FIB.

Results from indirect fluorescent antibody analysis indicated that purulent material from induced and natural lesions contain antigens that bind antisera produced against isolates RB29, and NB4H from diseased oysters. Lack of detection of individual bacteria may be due to release of soluble antigen into tissue surrounding bacterial colonies. This material could account for the brightly fluorescing foci found in the purulent material tested to date. Similar findings of large amounts of soluble antigen have been noted in other infections such as bacterial kidney disease of salmonids caused by *Renibacterium salmoninarum* (Hoffman et al., 1989).

Bacterial Identification The morphology, staining properties, and catalase reaction indicate that the bacterium isolated from diseased oysters, like that observed within infected tissues, is an actinomycete. All seven isolates from diseased oysters are slow-growing, gram-positive, and acid-fast; have a trilaminar Lechevalier type IVA cell wall *chemotype*; contain nocardomycolic acids with 44 and 58 carbon numbers; and contain a DNA base composition average of 68.80 mol% G + C. These characteristics indicate that the seven bacterial isolates from diseased oysters are homogeneous and, perhaps, identical and belong in the genus *Nocardia* (Lechevalier, 1986, Kudo et al., 1988). *Nocardia* are ubiquitous, aerobic, soil saprophytes and are often found in decaying material such as wet straw or hay (Ferry et al., 1988). However, some species are facultative or, less frequently, primary pathogens of humans, fish, and many mammals (Beaman 1973, 1984; Kusuda and Taki, 1973; Kudo et al., 1988). Nocardial infections are predominantly systemic and are generally considered to be opportunistic (Davenport and Johnson, 1986). *Nocardia* is a slow-growing bacterium characterized by filamentous, branched mycelia (>1 μm in diameter) that are nonencapsulated and non-spore–forming and usually fragment into coccoid or bacillary forms. The bacterium is gram-positive or gram-variable, weakly acid-fast, and PAS positive (Lechevalier, 1986). These characteristics are consistent with our findings and may indicate a new nocardial pathogen.

Like other species of *Nocardia*, the bacterium isolated from diseased oysters contained galactose, arabinose, and, to a lesser extent, glucose as the major cell wall sugars. The base composition of DNA from the bacterium isolated from diseased oysters most closely resembled that of *N. seriolae* (68.80 and 69.54 mol% G + C, respectively). However, the DNA base composition was also close to that of the other nocardial DNA tested. The base composition of DNA from various nocardial species was similar to that determined by Kudo et al. (1988) and reported by Lechevalier (1986) for several nocardial bacteria.

Results from biochemical tests indicate that although the *Nocardia* isolated from diseased oysters is similar to *N. seriolae* and, to a lesser extent, to *N. asteroides* GUH-2, it is unique in its inability to grow on most media tested. These findings were also reported by Kudo et al. (1988) for *N. asteroides* and *N. seriolae*. Immunofluorescent studies by Friedman and Hedrick (1991) showed that *Nocardia* sp. isolated from diseased oysters shared some common antigens with several nocardial species examined, including *Nocardia amarae*, N. asteroides, *Nocardia brasiliensis* and *N. seriolae*, and not with non-nocardial species such as *Vibrio anguillarum* and *Yersinia ruckerii* (two pathogens of fish; Post 1987). Antigenically, the oyster isolates were most similar to *N. asteroides* and least similar to *N. brasiliensis*.

On the basis of the morphological, antigenic, and biochemical properties; mycolic acids of the *Nocardia* sp. from oysters; and DNA base composition analyses, we propose that the bacterium isolated from oysters represents a new species of *Nocardia*: *Nocardia crassostrae*. DNA hybridization studies are currently in progress to determine the ultimate taxonomic relationship of this bacterium and other nocardial pathogens of marine organisms.

Cooperating Organizations
Battelle Marine Research Laboratory
California Department of Fish and Game
Coast Oyster Company
Department of Fisheries and Oceans, Canada
Johnson's Oyster Company
Westcott Bay Seafarms

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Fisheries

Age Determination of Bank Rockfish: Comparison of Traditional and Computer-Aided Techniques

Moss Landing Marine Laboratories
University of California, Davis
R/F-113
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Gregor M. Cailliet and Louis Botsford

Many of the 63 species of rockfish (genus *Sebastes*) found in the northeastern Pacific (American Fisheries Society, 1980) are an important resource for commercial and recreational fisheries (Proceedings of the International Rockfish Symposium, 1987). The genus is characterized as long-lived; some species have attained confirmed ages in excess of 60 years (Beamish, 1979; Bennett et al., 1982; Campana et al., 1990). This long life span presents unique challenges with respect to the management of species within the group.

In California, the advent of deeper water trawling gear since the early 1970s resulted in increased catches of certain *Sebastes* species (Lenarz, 1987). Approximately 2000 metric tons of bank rockfish (*Sebastes rufus*), caught at depths of 90 to 220 fathoms, are currently landed each year. This ranks it among the top five species in central California rockfish landings. Little is known about the biology of bank rockfish. Miller and Lea (1972) list its distribution from Guadalupe Island, Baja California, to off the Mad River in northern California, although it occurs in Oregon landings (Richmond, personal communication). Recently, Escheverria (1987) and Love (in press) included bank rockfish in their studies of rockfish reproduction, but age-specific information was limited because of difficulties with age determination. Thus, information on the age composition and growth of this species, vital for its management, is lacking. Bank rockfish otoliths have been collected from central and northern California commercial landings since 1977 and were made available to this project by the National Marine Fisheries Service (NMFS) and the California Department of Fish and Game (CDFG).

Traditional methods for determinating the age of otoliths are time-consuming and subjective, and in the past, the results have been erroneous (Beamish, 1979, Cailliet et al., 1986a). More objective approaches to this problem have been developed (Casselman 1983), including computer-aided image analysis. Systems of this type have been used successfully for shorter lived species such as the salmonids (Cook and Lord, 1978; Frie, 1982, 1985; Small and Hirschorn 1985a, 1985b), but had yet to be successfully used for rockfish. Our intention was to develop such a system at the University of California, Davis (UCD), and compare it with traditional aging methods at Moss Landing Marine Laboratories (MLML), while at the same time researching the age and growth of the bank rockfish.

The specific objectives of this study were to (1) collect, catalog, and subsample otoliths and their corresponding data from the archives at NMFS, Tiburon, and CDFG, Menlo Park; (2) train three readers to use currently accepted traditional methods of determining age, and use these readers to evaluate the traditional techniques by using variability indexes; (3) experiment with band (concentric growth zones on the otoliths) elucidation techniques for both traditional and computer-aided methods of determining age; (4) examine changes in otolith morphology associated with age; (5) develop computer-aided techniques for determining age and compare them with traditional methods; (6) use the computer-aided technique developed to determine the age of yearly subsamples and use these data to work up a cohort analysis of the *S. rufus* population; and (7) assess the annual periodicity of band formation, using edge analysis as one validation method.

Approximately 8000 bank rockfish were sampled from commercial landings from 1977 through 1988 as part of an ongoing cooperative groundfish sampling program between NMFS and CDFG. Samples were collected at ports from Avila to Eureka. Two 50-lb clusters were randomly selected from each landing. The species of each individual was determined, and the total length (in millimeters), sex, and the presence or absence of eyed larvae in females were recorded. Otoliths were removed, cleaned in running water, and stored dry in coin envelopes.

These data and otoliths were retrieved and cataloged into a database program on the MLML MicroVax computer. A subsample of otoliths from representative size classes (small, medium, and large) from 1987 was used to assess traditional methods of determining age. "Average Percent Error" (Beamish and Fournier, 1981) was used as an index of variability among age estimates. Another subsample, with representatives from all years, was selected for use in the cohort analysis.

Both Sea Grant trainees, Diana Watters and Aaron King, were trained by Tina Escheverria and David Woodbury (NMFS, Tiburon, California). We consulted with other leading experts in the field of rockfish age and growth, such as Shayne MacClellan (Pacific Biological Station, Nanaimo Bay, British Columbia), at the 1988 Committee of Age Reading expert meetings in Seattle. A third age reader, used in the part of this study that evaluated the traditional methods of determining age, was trained by the trainees. As work on the traditional

methods progressed, definite changes in the learning curves for reading errors were observed. This led to an abandonment of all initial readings until a level of experience was achieved that corresponded with lower variance within and between readers.

We have concluded that reading whole otoliths is unreliable, because the other two methods resulted in significantly higher counts, as found in other studies on the determination of age in rockfish (Chilton and Beamish, 1972). Use of thin-sectioned and "break and burn" otoliths produced comparable results. Thin sectioning of samples, along with polishing to elucidate the bands, was the most time-consuming of the three techniques; however, it provided a flat surface, which was more conducive to computer-aided image analysis. We experimented with other methods for elucidating the bands. These included baking the otoliths to "preburn" them before sectioning. We thought that this method could combine the positive aspects of break and burn with those of thin sectioning. Although we had only limited success with this method, we think further research along these lines could be beneficial. Methods of otolith staining (Albrechtsen, 1968; Bouain and Siau, 1988) were attempted, but none were successful.

The most promising technique for computer-aided image analysis involved mounting thin sections of otoliths on microscope slides, grinding them down to permit light to pass through them, polishing their surfaces, and applying immersion oil. This thin-section polishing technique is the one we used to process the subsample of 3000 otoliths for computer-aided determinations of age to be used in the cohort analysis. At our suggestion, Buehler Ltd., a company that markets equipment used in thin-sectioning techniques, is also evaluating the state of fish otolith thin sectioning. Its goal may be to market techniques for otolith thin sectioning that require use of their equipment.

Otolith morphometrics (length, width, surface area, curvilinear length, average radius and perimeter) were measured by using an Olympus Cue II image-analysis system, and otoliths were weighed. These data will be compared with age estimates to determine if significant correlations exist that could also be used for age estimation and to look for inflection points in the species' growth curves.

The group at UCD (Botsford, Brittnacher, Kope, Ford, and Matsubayashi) worked primarily on development of the hardware and software for the computer-aided age determination. This system was developed to speed up the otolith-reading process and maintain consistency from otolith to otolith. While doing so, it maintains involvement of the human reader by leaving to the reader both decisions on the initial set-up of system parameters and the final decision on each identified mark. The hardware used is an Apple Macintosh II with a microscope, video camera, video monitor, and frame grabber. The Macintosh system was chosen because of the inherent ease with which the user can deal with graphics and images.

The operation is as follows. The otolith is placed on the microscope stage, and all adjustments to focus and location are made by viewing the image on the video monitor. When the operator is satisfied with the image, the Macintosh is told to grab a frame from the video camera and display the image on the Macintosh screen. The operator then selects a rectangular region running transverse to the parallel, periodic bands that are to be counted. The light level is then scanned along a preset number of parallel lines within the rectangle. The first step in processing is to average these parallel lines across the width of the rectangle. However, as that width increases, the dark and light bands may not line up, and a type of phase or registration error can occur.

The next step in processing is to further reduce noise by filtering the one-dimensional signal that results from the averaging. This is done by using either (1) a Fourier transform or (2) a matched filter. A Fourier transform is performed on the signal to reduce it to a sum of sinusoids. Those sinusoids near the spatial frequency of the parallel marks being read are then selected, and the Fourier transform is then inverse transformed to create the filtered signal. The matched filter method is based on the fact that the best way to detect a known signal in white noise is to correlate the noise plus signal with the known signal. When they "match," the signal is considered detected. In our case, the signal is the shape of the annual (or daily) mark on the otolith. Our matched filter is implemented by numerical convolution. Both of these filters are followed by a peak or valley detector that identifies the annual marks.

The result that the operator sees on the screen is a hash mark designating each point the system has identified as an annual mark and a count of how many marks occur in the region chosen (Figure 1). When the matched filter is used, this occurs almost immediately after the rectangular region is selected; but when the Fourier transform is used, it occurs after approximately 1–3 minutes. The operator may then change any of the decisions for the final reading. Information from any readings can be placed automatically or manually into an editable test window to produce a report of the results.

The trainee at Davis (Matsubayashi) has pursued further automation with two-dimensional Fourier transformation and the use of simple artificial intelligence schemes to reduce the number of false valleys.

We are in the process of comparing performance of this system with that of a human reader alone.

Assessment of the annual formation of opaque and translucent bands by using "edge analysis," a method of age validation (Mayo et al., 1981; Beamish and McFarlane, 1983), is in progress. Preliminary results indicate that these bands are deposited annually; opaque bands form from about June through October and translucent bands from about November through May. Otolith thin sections from approximately 150 specimens less than 310 mm in total length are being used for this analysis, as edge type is

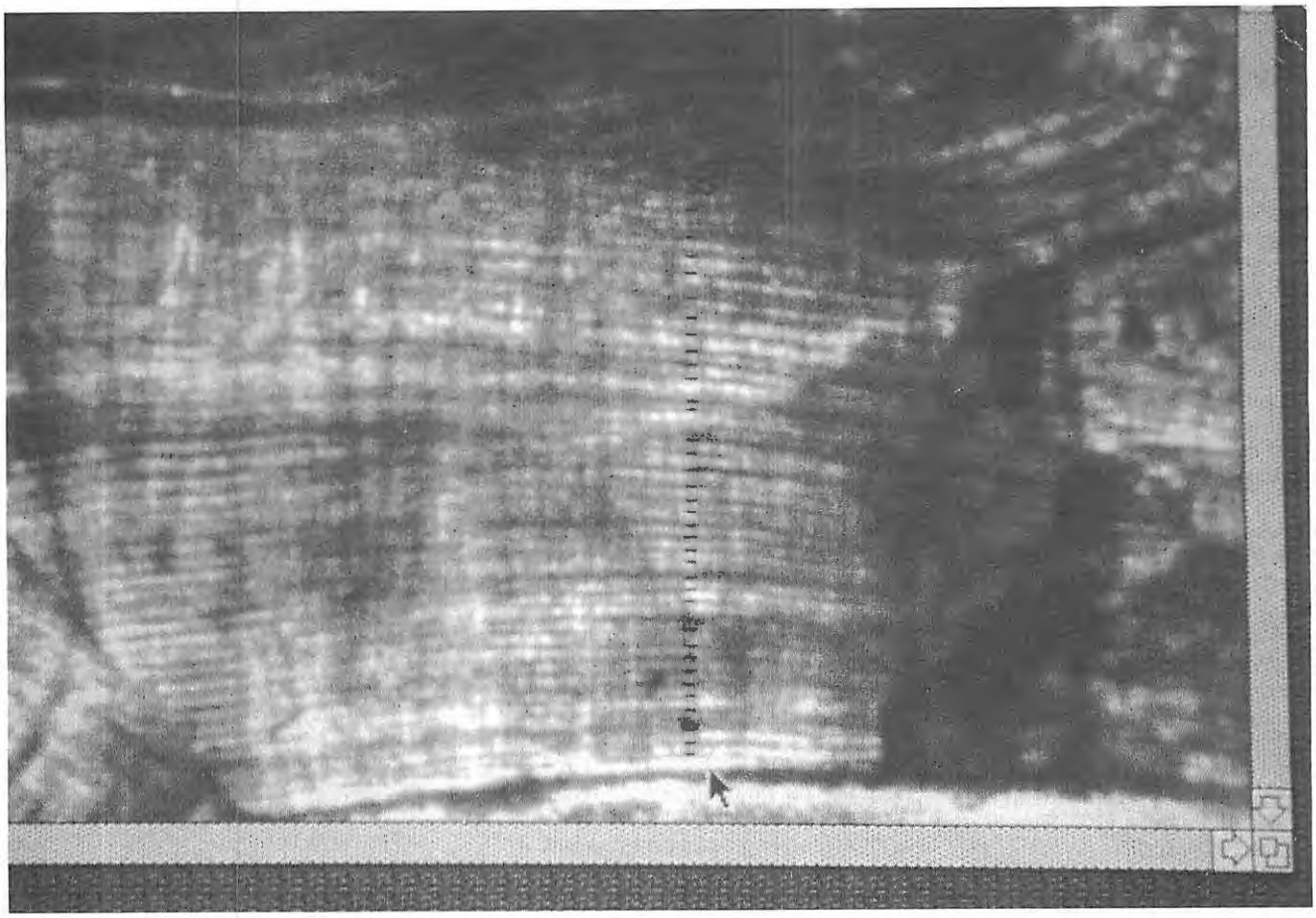

Figure 1. Hash marks designate each point the system has defined as an annual mark.

not discernable in larger specimens. Because we lacked a sufficient number of specimens of this size, additional specimens were obtained from Dr. Milton Love of the University of California, Santa Barbara. These were randomly collected from area fish markets.

For a more objective assessment of opaque and translucent band formation, we used electron microprobe analysis. This method has been used with some success to evaluate growth patterns in elasmobranch centra (Jones and Geen, 1977; Cailliet et al., 1986b; Cailliet and Radtke, 1987) and in other structures used in age determination of teleosts (Casselman, 1974; Cailliet et al., 1986a, Radtke et al.,1988). It had not been attempted for rockfish. We collaborated with personnel at UCD's geology department, where analyses for calcium, carbon, and other elements were conducted using a Cameca 50X microprobe. Calcium content along growth axes in bank rockfish otoliths revealed patterns that did not correlate well with the visual patterns used in age determination, although a highly significant difference in calcium concentration was found between opaque and translucent zones. The results of this work were presented as abstracts at the 1989 California Cooperative Fishery Investigations and American Society of Ichthyologists and Herpetologists annual meetings.

The expected benefit of our work with computer-aided techniques is a clear idea of the suitability of this technique for determining the age of rockfish otoliths and, if the techniques work adequately, more objective estimates of age for rockfish. Our results from the cohort analysis now being done will enable federal and state bank rockfish managers to determine if past and present fishing pressures have had any detrimental effects on the population. As the use of computer-aided techniques for determining age and edge-analysis techniques for validating age are relatively new to fishery management, ongoing studies such as this one may eventually lead to industry-wide routine management techniques that include these methods.

We were expecting to be finished with all aspects of this study by the end of the 1989 calender year. However, the earthquake and other events have delayed completion. In addition, we are using radiometric

age verification techniques (R/NP-1-20D) to obtain independent estimates of bank rockfish ages (See Campana et al., 1990).

Cooperating Organizations

Buehler, Ltd.
California Department of Fish and Game, Menlo Park
California State University, Fresno, Geology Department
Committee of Age Reading Experts
Myers Foundation
National Marine Fisheries Service
Packard Foundation
University of California, Davis, Geology Department
University of California, Santa Barbara
University of California, Santa Cruz

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Age-specific Analysis of Rockfish Fisheries

University of California, Davis/
California Department of Fish and Game
R/F-114
Project Initiated: October 1, 1988
Project Completed: September 30, 1989

Louis W. Botsford and Frank D. Henry

Bocaccio, *Sebastes paucispinis*, and chilipepper, *S. goodei*, are two of the most important commercially fished rockfish species in California. In most years since 1978, bocaccio and chilipepper have ranked first and second in weight of rockfish landed at most California ports. The bank rockfish, *S. rufus*, is also rapidly increasing in importance and is among the top five in central California landings.

Current management of rockfish fisheries is based on catch quotas estimated from yield-per-recruit analysis with parameters determined by catch-age analysis (Henry, 1986). These analyses have been relatively successful for chilipepper, but problems have been encountered with bocaccio, and bank rockfish otoliths have not yet been aged. Analyses for all three species require more effort in description and evaluation of the error structure in catch-age analysis. The yield-per-recruit analysis could explicitly account for effects on reproduction. Also, the propagation of errors and effects of all sources of uncertainty on management recommendations should be explicitly evaluated. Ongoing developments in analytical techniques and the availability of age data on bank rockfish (Sea Grant project R/F-113) provide the opportunity to develop better strategies for management of these three species.

The overall objective for this project was to improve the management of chilipepper and bocaccio fisheries by developing better estimates of population parameters, an improved understanding of the accuracy of these estimates, and an analysis of the implications of uncertainty for management of these fisheries. Originally this was to be accomplished by developing a catch-age analysis that included a more-accurate description of errors (e.g., Fournier and Archibald, 1982; Methot, 1986) than the catch-age analysis presently used by the California Department of Fish and Game (CDFG) for parameter estimation (Deriso et al., 1985; Henry, 1986). Parameters estimated using this catch-age analysis model were to be used to perform a yield-per-recruit analysis that takes egg production into account (e.g., Botsford and Hobbs, 1986) to examine how optimal management changes as the importance of reproduction changes. Recruitment estimates were also to be used to investigate the influence of the marine environment on reproductive success in the chilipepper and bocaccio populations.

We assembled age-structured catch data for chilipepper and bocaccio fisheries by quarter and by port area for all years that data are available. Both species have sexual dimorphism, and separate catch estimates are available for each sex. Cursory examination of these data and discussions with fishery scientists at the National Marine Fisheries Service and the CDFG led us to believe that variability in the spatial distribution of the fishery and the population should be detectable in the catch data, and we believed also that there should be some way to take advantage of this spatial component in the analysis of the data. To this end, we developed a spatial catch-age analysis model that fits the parameters of a spatial fishery model (based on diffusive movement of fish) to spatially structured catch-at-age data using a chi-square objective function and steepest-descent fitting procedure similar to that of Kope (1987).

We tested and evaluated this spatial catch-age analysis methodology using simulated data generated with the spatial fishery model. We found that, although information about the spatial distribution of recruitment and population density would be very valuable in formulating management policy, if realistic levels of error are present in the catch data, it is not possible to estimate spatial parameters reliably. In addition, performing the spatial catch-age analysis is so computationally intensive and time consuming that it would not be useful for routine data analysis on personal computers. However, using simulated data generated with the spatial fishery model, we were able to evaluate the application of the traditional catch-age analysis to spatially structured data and compare the results with those obtained using spatial catch-age analysis. Results of this evaluation include the following:

(1) Conventional catch-age analysis techniques that ignore spatial aspects of the fishery can provide better estimates of mortality rates, recruitment, and age-specific vulnerability than the more realistic spatial catch-age analysis.

(2) Conventional catch-age analysis of data from all ports lumped together provides recruitment estimates that are comparable to, or better than, estimates obtained by analyzing data from each individual port separately, even when there is no movement of fish between ports.

(3) Accurate description of the error structure in the catch data is less important in obtaining stable parameter estimates than is the inclusion of auxiliary information (e.g., fishing effort or independent abundance estimates).

(4) When effort data are included as auxiliary information, the most effective method of combining data from several ports is to weight the density of fishing effort for each port area by the relative population abundance in that port area.

We are presently preparing a manuscript detailing our development, testing, and evaluation

of spatial catch-age analysis.

The implications of the above findings are that the best way to analyze the data for chilipepper and bocaccio is to combine the data for all ports and that it is almost mandatory to include auxiliary data to obtain reasonable estimates. The former is possible, but auxiliary data for the chilipepper and bocaccio fisheries are virtually nonexistent. Because chilipepper and bocaccio are fished with the same gear and have similar distributions, we developed a multispecies catch-age analysis model to use data for both sexes of both species simultaneously. Separate catch data for both sexes have been used, in conjunction with other auxiliary data, to generate synthetic stock estimates for sablefish, *Anaplopoma fimbria* (Methot and Hightower, in prep.). We believed that redundancy in the four data series (two sexes and two species) from what is essentially a single coherent fishery can be exploited to yield information about historic levels of fishing effort and that this method would stabilize recruitment estimates nearly as well as actual auxiliary effort data. This multispecies catch-age analysis did provide better estimates of fishing effort than the conventional single-species analysis, but it did not result in better estimates of recruitment as expected. Consequently, we confined further investigations into the propagation of errors in catch-age analysis to the conventional single-species type of analysis that is presently used on rockfish.

To perform the error analysis, we developed a computer program to efficiently estimate recruitment, fishing effort and age-specific vulnerability using a chi-square objective function; this program also allowed us to include auxiliary effort data. We used this model to analyze artificial sets of catch-at-age data with known error distributions, and we then examined the performance of the resulting parameter estimates. The results were somewhat surprising. When catch-at-age data had observational errors that were on the order of 10% (coefficient of variation, CV), recruitment was estimated with a CV on the order of 50%–250% in the absence of auxiliary information. If auxiliary effort data with reasonable estimation errors (10% CV) were included, this was reduced to a CV of 5%–30% in the recruitment estimates with 10 years of data. We tried a number of strategies to constrain recruitment estimates in the absence of auxiliary data. The strategy that worked best was to assume that fishing effort had been constant and to incorporate this constant as auxiliary data. This strategy produded a CV in recruitment estimates that increased from about 7% to about 40% over the 10 years in which recruitment was estimated. While this is encouraging in that it is possible to constrain solutions obtained in the absence of auxiliary information to produce estimates that are nearly as accurate as solutions obtained with auxiliary information, these recruitment estimates are not precise enough to warrant their use for investigation of environmental influences on recruitment with a time series that is only 10 years in length.

We have obtained all the necessary data and have developed the computer software necessary to perform a graphical analysis of the consequences of management strategies on population egg production. The software we have developed also allows for the solution of optimal management under prescribed constraints on population egg production and for various fishing costs. We anticipate that these analyses will be completed soon.

Cooperating Organizations

California Department of Fish and Game, Menlo Park

National Marine Fisheries Service, Tiburon

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Description of the Larval Development of Field and Laboratory Grown California Rockfish (*Sebastes*) Species

Moss Landing Marine Laboratories
R/F-115
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Valerie Loeb and Gregor Cailliet

The objective of this project was to establish developmental series, through grow-out studies, of various common central California rockfish (*Sebastes*) species whose larval stages were undescribed or partially described. The resulting descriptions of early life history will enable identification of field-caught specimens and, with associated information on adult reproductive potential and behavior, will aid fisheries research directed toward understanding spawning stock-recruitment relationships.

Knowledge of the stages of early life of eastern Pacific rockfish species has been limited by problems associated with rearing the larvae from known adults, problems that are partly due to the difficulty in obtaining naturally extruded, full-term larvae from field-caught females. Other problems are associated with feeding the relatively small and undeveloped early larval stages. One of our major project objectives was to establish techniques and procedures that would permit successful rearing of larvae from central California rockfish species. The likelihood of our success in this objective was enhanced by the opportunity to establish culturing operations in the new, modern research facility of the Monterey Bay Aquarium where we could depend on filtered seawater and controlled light conditions essential for developing algal and rotifer cultures at sufficient densities for successful feeding by the larval fish. At the Aquarium, we had access to fish-transportation equipment and holding tanks for field-caught brood stock, as well as display tanks for additional sources of brood stock. These features made it possible to maintain pregnant females in captivity and thereby greatly increased the probability of nonstressed, full-term parturition.

Our second major objective was to describe the larval developmental series by using established techniques, including descriptions of morphometrics and pigment distributional patterns at different sizes and stages of development. The species we targeted for study are primarily shallow-living as adults and are important in recreational and commercial fisheries. These included the blue rockfish (*S. mystinus*), kelp rockfish (*S. atrovirens*), gopher rockfish (*S. carnatus*), black-and-yellow rockfish (*S. chrysomelas*), China rockfish (*S. nebulosus*), grass rockfish (*S. rastrelliger*), and treefish (*S. serriceps*). Within this group are closely related species pairs (e.g., *S. carnatus* and *S. chrysomelas*), and it was important to determine whether the larvae may be distinguished at the species level. Two deeper-living species, the chilipepper (*S. goodei*) and yellowtail rockfish (*S. flavidus*), were also targeted because of their commercial importance.

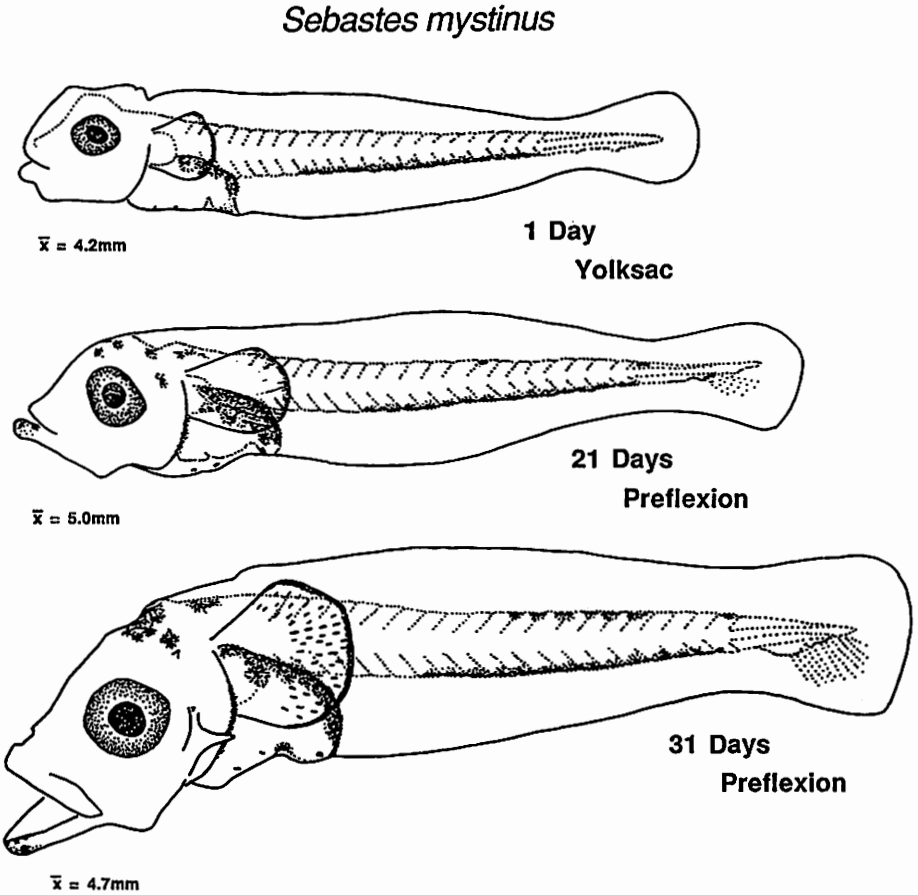

Figure 1. Early life stages of the blue rockfish, *Sebastes mystinus*, based on laboratory-reared larvae. $\bar{x}$ = mean length.

In the first few months of the project, algal- and rotifer-culturing operations were set up, two larval fish-rearing tanks at the Aquarium were refitted and installed. First field-collection efforts in November and December of 1988 involved both hook-and-line fishing and SCUBA-diver–mediated techniques. It was soon discovered that the SCUBA divers could readily identify pregnant females of target species and could selectively catch them using baited hooks and gill nets. These capture methods and subsequent hand transport to holding tanks appeared to produce minimal stress in the animals and did not result in premature parturition, as has been reported for hook-and-line capture. Because of its relative efficiency and low-stress results, sample collection by SCUBA divers herding selected fishes into small underwater gill nets became our primary source of shallow-water species during 1989.

During 1988, larvae were obtained from field-collected, pregnant *S. mystinus, S. chrysomelas*, and *S. carnatus* and from pregnant *S. atrovirens* females removed from the Monterey Bay Aquarium Kelp Tank display. In 1989, additional broods were reared from field-caught *S. mystinus, S. carnatus*, and *S. atrovirens* females and from a pregnant *S. rastrelliger* removed from the Kelp Tank. *Sebastes melanops* larvae were obtained from a field-caught female that gave birth at the Telonicher Marine Laboratory (California State University, Humboldt).

During our attempts to obtain viable full-term larvae from the field- and tank-caught females, we became aware of the importance of timing in collection efforts. The females must be sufficiently advanced in pregnancy so that resorption of larvae cannot occur (e.g., they must be past a "point of no return"). However, if the females are collected too near to parturition, larval development can be adversely affected or the larvae prematurely extruded. On the basis of our observations, the optimal collection period appears to be about 3 weeks before parturition. However, this time period and resilience to capture may vary with species.

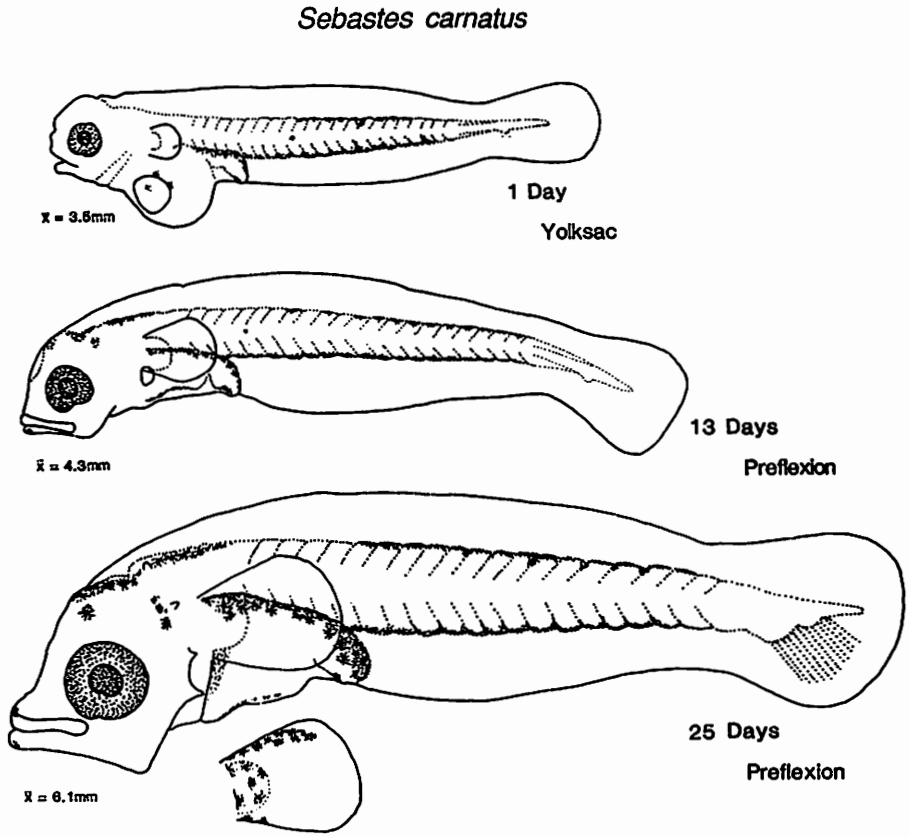

Figure 2. Early life stages of the gopher rockfish, *Sebastes carnatus*, based on laboratory-reared larvae. $\bar{x}$ = mean length.

Poor larval survival rates (e.g., <12 days) during the initial rearing attempts and subsequent postmortem examinations of the larvae indicated starvation as the most likely cause of death. To support greater food densities, we doubled our algal production and scaled up our rotifer cultures from 5-gallon carboys supplied solely with algae to 30-gallon containers supplied with algae and a lipoprotein food supplement developed by Hubbs Marine Research Institute. We included wild zooplankton with the rotifers to increase food densities and to provide a variety of alternative food items. These efforts resulted in increased survival rates (e.g., 15–31 days) and apparently improved larval feeding, growth, and development in the later 1988 rearing projects. During 1989, the daily introduction of freshly caught, filtered zooplankton to rotifer-*Artemia* food sources increased the overall length of survival to 54 days. Problems with high larval mortality during the second year were associated with attempts to rear larvae in smaller tanks than in 1988 (130 vs. 400 liters) and also to poor early larval survival despite the augmented food supplies.

During the rearing operations, larvae were removed from each brood at approximately 4-day intervals, photographed, and preserved for subsequent descriptive analyses. Morphometric measurements were done at the Moss Landing Marine Laboratories using an Olympus image-analysis system. Pigment patterns were mapped according to a modification of the scheme presented by Kendall and Lenarz (1987). We added seven areas of pigment concentrations to the 26 previously described loci. We also produced a scheme of describing age- and growth-related development of larval pigmentation

by using the proportions of larvae within set size ranges that demonstrated pigmentation at each locus instead of the previously used pigment presence/absence scheme. Illustrations were made of available developmental stages for each species using projected photographic slides and the pigment patterns from preserved larvae.

Detailed results of the rearing projects and larval descriptions (pigmentation patterns, morphometrics) are presented in Master's theses by the Sea Grant trainees (Moreno, 1990; Wold, 1991). Characteristics of the six species are briefly outlined here.

Sebastes mystinus. Gravid females were collected from December to mid-February, with parturition occurring from January to mid-March. A total of 10 females gave birth, each producing at least two batches, with 3- to 5-day breaks between batches. This species was the most difficult to rear. Many broods were obtained and rearing attempts made, but in most cases little or no growth was observed, probably because of starvation. The larval size range was from 3.2 mm at birth to 5.5 mm at day 31 (maximum survival). None of the larvae underwent flexion. The early larvae (Figure 1) are distinguished from those of the other five species by having relatively little pigmentation on the head and body but some pigmentation on the pectoral fin base and margins.

Sebastes melanops. One brood from a gravid female caught in February was reared at Telonicher Marine Laboratory. The larvae lived for 22 days but showed signs of starvation and no increased growth or development after yolk absorption. As a consequence, only larvae 1–3 days old were described. The larvae ranged in size from 3.4 mm at birth to 4.1 mm at day 3. Of the five species, this had the least amount of pigmentation and had a characteristic melanophore in the lower caudal fin lobe.

Sebastes carnatus. Gravid individuals were observed from March through late May. Three of four field-caught females gave birth to full-term larvae. Larval sizes ranged from 3.1 mm at birth to 6.2 mm at day 28 (maximum survival); none attained flexion. The early larval pigmentation patterns (Figure 2) are very similar to those of *S. chrysomelas* (Figure 3) and somewhat similar to *S. atrovirens* and *S. rastrelliger* (Figures 4 and 5, respectively). Larger larvae of the four species can be easily differentiated by the number of melanophores occurring in various head and dorsal trunk regions (loci 4, 6, 18, and 19). *Sebastes carnatus* is further distinguished from the other species by pigmentation on the base and upper margin of the pectoral fin.

Sebastes chrysomelas. Pregnant individuals were observed in the field and collected during March. Larvae were obtained from three parents; they ranged in size from 4.4 mm at birth to 5.6 mm at 23 days (maximum survival). One larva reached flexion at 21 days of age. The early larvae (Figure 3) are indistinguishable from larvae of closely related *S. carnatus*; the larger stages are distinguished by the absence of pectoral fin pigment and the persistence of the lateral trunk melanophore present in the early stages.

Sebastes atrovirens. Larvae were obtained from two females removed from the Monterey Bay Aquarium Kelp Tank. One female produced premature larvae and unfertilized eggs at capture; the other gave birth to full-term larvae. The full-term larvae were 4 mm at birth and grew to 6.5 mm by day 30 (maximum

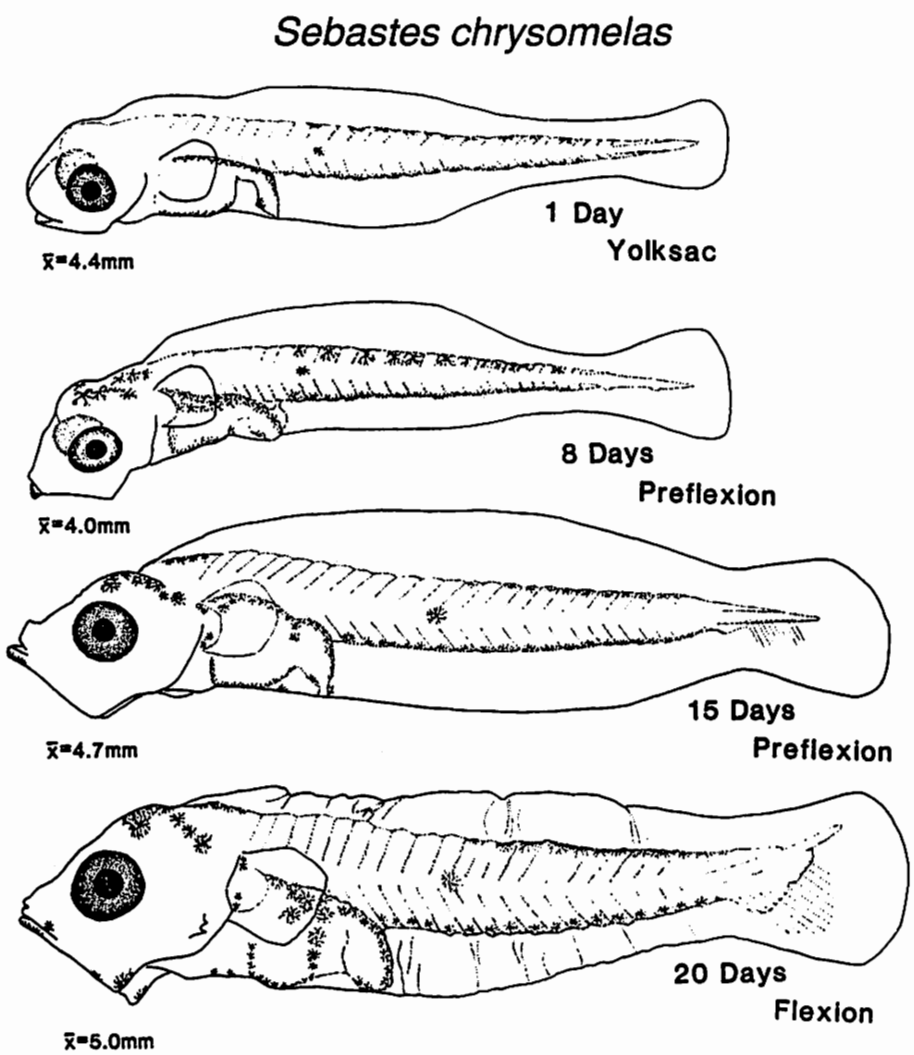

Figure 3. Early life stages of the black-and-yellow rockfish, *Sebastes chrysomelas*, based on laboratory-reared larvae. $\bar{x}$ = mean length.

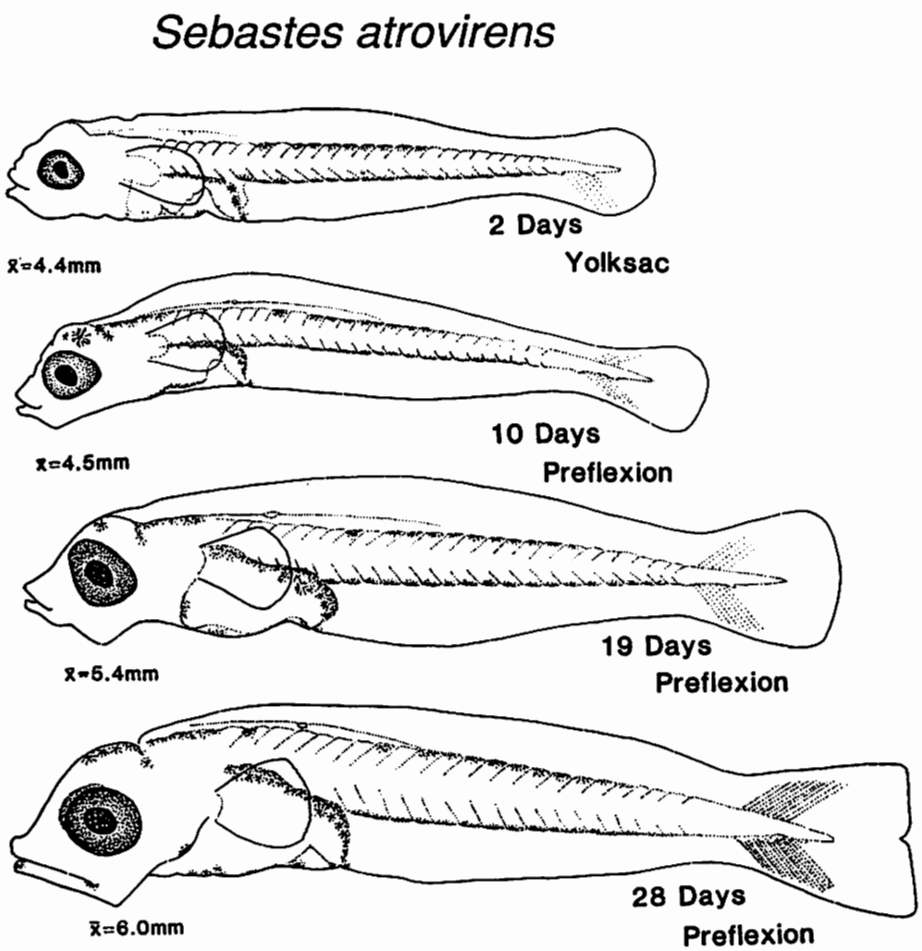

Figure 4. Early life stages of the kelp rockfish, *Sebastes atrovirens*, based on laboratory-reared larvae. $\bar{x}$ = mean length.

survival) (Figure 4). These larvae lacked the pectoral fin pigmentation and the lateral trunk melanophore characteristic of *S. carnatus* and *S. chrysomelas* and had less dorsal pigmentation than *S. rastrelliger*.

Sebastes rastrelliger. One gravid female was collected from the Aquarium Kelp Tank. The larvae were released at capture but appeared to be full term (without yolk sacs or oil globules). They ranged from 4.3 mm at birth to 7.7 mm after 52 days. After 54 days, the rearing was terminated because of the small number of live larvae left. The larvae went through flexion and to the beginning of obvious dorsal and anal fin development (Figure 5). At all stages these larvae were the most heavily pigmented of the six species and thereby readily identifiable.

Cooperating Organizations

California Department of Fish and Game, Monterey and Granite Canyon
California Edison Research and Development Laboratory, Occidental College
California State University, Humboldt, Telonicher Marine Laboratory
Hubbs Marine Research Institute
Humboldt State University
Monterey Bay Aquarium
National Marine Fisheries Service, La Jolla, Bodega Bay, Alaska and Honolulu laboratories
University of California, Santa Barbara
University of Washington Friday Harbor Laboratory
Vancouver Public Aquarium

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Wold, L. 1991. A practical approach to the description and identification of *Sebastes* larvae. Master's thesis, California State University, Hayward.

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Moreno, G. 1990. Description of the larval stages of five northern California species of rockfishes (Family Scorpaenidae) from rearing studies. Master's thesis, California State University, Stanislaus.

Moreno, G. 1992. In prep. Descriptions of early larvae of four northern California species of rockfish (Family Scorpaenida) from rearing studies. NOAA Technical Report.

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Wold, L., G. Moreno, G. M. Cailliet, and V. J. Loeb. 1988. Descriptions of the early developmental stages of shallow water rockfish (*Sebastes*) from central

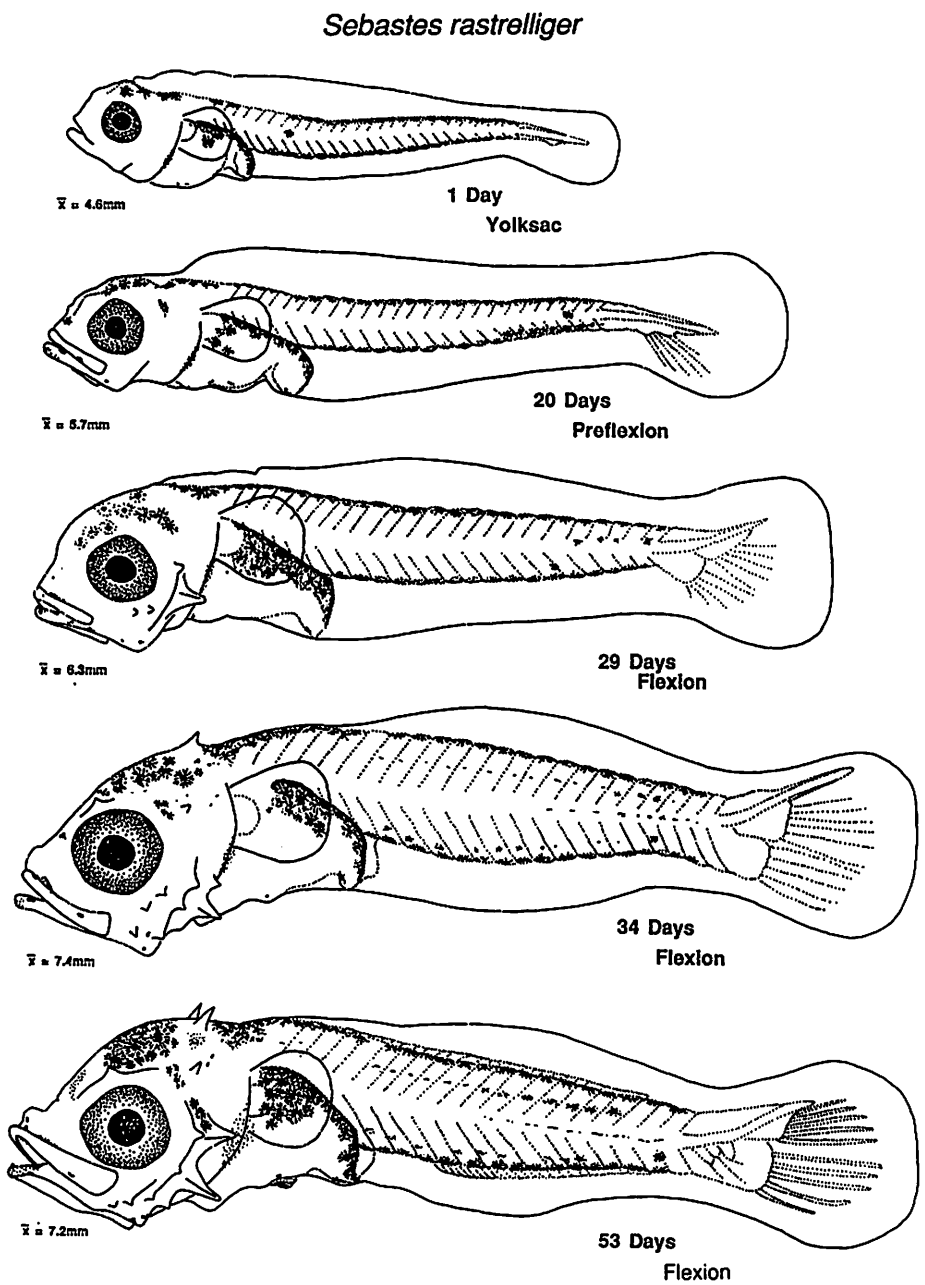

Figure 5. Early life stages of the grass rockfish, *Sebastes rastrelliger*, based on laboratory-reared larvae. $\bar{X}$ = mean length.

California. Poster presentation. 75th Meeting of the American Association of Ichthyologists and Herpetologists, Ann Arbor, Michigan, June 1988.

Collagenolytic Activity in the Skeletal Muscle of Fish

University of California, Davis
R/F-119
Project Initiated: October 1, 1987
Project Completed: September 30, 1990

Norman F. Haard

Introduction

After postmortem rigor has disappeared, the texture of fish muscle tends to become soft during storage at temperatures above freezing. This postmortem change may be caused by a variety of factors. Examples include release and activation of proteolytic enzymes from protozoan parasites, rapid catabolism of ATP resulting in a relatively low ultimate pH before the carcass is chilled, and seepage of digestive enzymes from the belly cavity to the flesh. Several workers have suggested that enzymic catabolism of collagen may be an additional factor. However, before this project, no direct evidence linked collagen degradation to softening of fish muscle. Collagen, the major constituent of connective tissue, plays an obvious part in maintaining the mechanical strength and integrity of muscle.

The twelve known types of collagen share a general structure consisting of three polypeptide units (α-chains) that together form a long triple-helical structure and shorter non-triple-helical domains. Intramolecular aldol cross-links and intermolecular aldimine and oxoimine cross-links between α-chains result in formation of dimer (β) and trimer (γ) units as well as other high molecular weight aggregates. Type I collagen, a fiber-forming collagen, is distributed in all structural levels of muscle tissue. Types III, IV, and V have also been detected in muscle tissue (Bailey and Light, 1989). Fiber-forming collagen self-assembles to form fibrils where these rodlike molecules stack over one another, by one-fourth their length in a staggered pattern. The mechanical strength and solubility of collagen and its susceptibility to enzyme-catalyzed hydrolysis is influenced by the organization of collagen molecules as fibrous structures. These same properties of collagen may also be influenced by the association of fibrils with noncollagen constituents of connective tissue, particularly the proteoglycans (Etherington, 1977). Proteoglycans are macromolecular carbohydrates that have a protein core to which glycosaminoglycan chains are covalently bound forming polymers of repeating disaccharide units of hexosamine and hexuronic acids.

Overview of Related Research

My colleagues and I hypothesized that endogenous collagenolytic enzymes are present in the muscle of fish where they contribute to postharvest softening of the flesh. To examine *in situ* degradation of collagen in muscle tissue, we developed an immunological method to detect collagen and its degradation products. Collagen degradation was monitored in carefully handled as well as in temperature-abused rockfish during storage of the fish on ice. Collagen chains remained intact in carefully handled, sterile, rockfish muscle stored on ice for up to three months. However, incubation of sterile rockfish muscle at 30°C resulted in disappearance of collagen chains. The catabolism of collagen that occurred in fillets incubated at 30°C was associated with a dramatic disintegration of the structure of muscle tissue. Two metalloproteinases were isolated from rockfish muscle. The enzymes had properties similar to those of mammalian collagenases, including the ability to catalyze hydrolysis of collagen fibrils. Possibly, these enzymes contribute to the degradation of collagen in fish that is not carefully handled after harvest.

In iced, sterile rockfish muscle, the solubility of muscle collagen increased during 1 week of iced storage. The increased solubility of collagen occurred concomitantly with softening of muscle texture. Species of rockfish (e.g., bocaccio) that did not soften much after harvest showed less increase in soluble collagen than species that tended to soften (e.g., yellowtail).

Two enzymes that may be involved with proteoglycan degradation, β-glucuronidase and β-N-acetylhexosaminidase, were detected in rockfish muscle. Proteoglycans were isolated from rockfish muscle, and an electrophoretic method was developed to monitor changes in iced fillets. Initial studies indicated that rockfish muscle proteoglycans are converted to lower molecular weight forms during storage of muscle tissue on ice for 7 days. Therefore, the increased solubility of collagen in iced rockfish muscle may be the result of degradation of proteoglycans.

Methods

Measurement of Muscle Texture. A shear-force method described by Lee (1983) was adapted to measure the muscle texture of raw rockfish. A detailed description of this method is presented elsewhere (Chou, 1989). It was necessary to carry out storage studies with fish of the same size (year class) because the raw texture of the flesh of large fish was firmer than that of smaller fish.

In some storage trials, samples showed gross loss in tissue integrity and tensile strength. The shear-force method was not useful for measuring the loss of fillet integrity in these samples (Chou, 1989). We measured the tensile strength of raw muscle by using an Instron method (Gillett et al., 1978). However, this method was of limited use for storage studies because of the number of samples rejected and the relatively large amount of tissue required. A uniaxial compression method (Christianson et al., 1985) was used to measure the texture of rockfish because the method required a relatively small sample size.

However, the method was not suitable for our studies because of poor reproducibility of replicates and lack of sensitivity to changes in gross tissue integrity (Kim, 1990).

Determination of Total Muscle Collagen. The total amount of collagen in fish muscle was estimated from the muscle's hydroxyproline content. The white muscle of fillets was freeze dried and ground to a fine powder, and 1-g samples were hydrolyzed in 6 N HCl (1:10 w/w) for 16 hours at 121°C. The amount of hydroxyproline in the hydrolyzed samples was measured by using the method of Leach (1960). For calculation of collagen, it was assumed that hydroxyproline was associated exclusively with collagen and that rockfish collagen contains 8% hydroxyproline (Bogason, 1984).

Extraction and Purification of Collagen. Collagen was isolated from carp skin by a method described by Yoshinaka et al. (1976) and was shown to be free from impurities by analysis on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). This protein was used as a collagen standard for SDS-PAGE.

Collagen was isolated from brown rockfish muscle by using classical techniques involving sequential extraction with neutral salt, dilute acid, and pepsin treatment (Miller and Rhodes, 1982). Collagen isolated from rockfish muscle by this method contained impurities as shown by amino acid analysis and SDS-PAGE. Also, the yield of collagen represented a relatively low percentage of the total hydroxyproline in muscle.

Collagen was isolated from the muscle of several rockfish species by using a modification of the alkali-insoluble protein method described by Kimura and Tanaka (1986) and by Sato et al. (1986). Recovery of hydroxyproline and results with SDS-PAGE and Western blots, with an antibody to collagen, showed that muscle collagen is completely recovered in the alkali-insoluble protein fraction. Acid-soluble collagen purified from alkali-insoluble protein was free of noncollagen proteins (Bracho and Haard, 1990; Cepeda et al., 1990). Collagen from rockfish muscle was used as a standard for SDS-PAGE and Western blots and was used to isolate α- and β- chains of collagen.

Acid-soluble collagen was extracted and purified from lingcod skin by the method described by Cawston and Murphy (1981). The final product was shown to be pure by SDS-PAGE and amino acid analysis. Collagen from lingcod skin was radiolabeled and used for assay of collagenase. The fish collagen was able to form fibrils at lower temperatures than mammalian collagen did.

Separation of Collagen Chains. Separation of collagen chains (α, β, γ) by ion exchange chromatography (Bogason, 1984) was not satisfactory. Therefore, α- and β-chains were cut from SDS-PAGE gels and used for Western blot analysis of individual chains and their hydrolytic products.

Cross-link Composition of Fish Muscle Collagen. The difunctional cross-link composition of muscle collagen from two rockfish species was determined by radiolabeling collagen with NaB^3H_4, hydrolyzing the labeled collagen with 6N HCl, and detecting cross-link residues by using high pressure liquid chromatography (Bracho and Haard, 1990). We studied collagen cross-linking because the solubility of the collagen of rockfish muscle was lower in older year classes of rockfish and varied with species of rockfish.

Measurement of Collagen Solubility. Soluble muscle collagen was measured by a procedure described by Hatae et al. (1986). Tissue was extracted with 0.1 N sodium phosphate, pH 7.0, at either 20°C or 70°C. The amount of collagen extracted was estimated on the basis of the hydroxyproline content of hydrolyzates. Preliminary studies showed that texture softening in several species of rockfish was associated with an increase in collagen soluble at 70°C; whereas the collagen soluble at an extraction temperature of 20°C did not change during storage on ice after harvest. All subsequent reference to soluble collagen in this report refers to material extracted at 70°C. Soluble collagen fractions were analyzed with SDS-PAGE and Western blots for the presence of degradation products.

Electrophoresis of Collagen. SDS-PAGE was done according to the method of Laemmli (1970). After electrophoresis of collagen chains on gels with different polyacrylamide concentrations and concentration gradients, we selected a 5–15% polyacrylamide gradient for optimal separation of collagen chains from one another and from noncollagen proteins. Gels were stained with Coomassie blue R such that collagen stained metachromically (pink) and noncollagen protein stained orthochromically (blue) (McCormick et al., 1979).

Immunodetection of Collagen and Its Degradation Products. Protein from SDS-PAGE was electrophoretically transferred to nitrocellulose membranes, and immunodetection was accomplished by using a monoclonal antibody to calf skin type I collagen. Detection of collagen on Western blots was facilitated by using a Vectastain ABC immunoperoxidase system (Vector Laboratories, Burlingame, California). The details of this method are described elsewhere (Cepeda et al., 1990).

Extraction of Proteoglycans. The white muscle of yellowtail rockfish was extracted at 4°C for 12–20 hours with 4 M guanidine chloride (10 v/w) containing 0.05 M sodium acetate, pH 5.8–6.0, and 0.1 M γ-aminohexanoic acid, 0.01 M EDTA, 1 mM benzamidine hydrochloride, and 10 mM N-ethylmaleimide as protease inhibitors (Oegema et al., 1975). The insoluble residue was removed by centrifugation (45,000 x g, 20 min.), and the supernatant was dialyzed against nine volumes of 0.05 M sodium acetate buffer, pH 5.8–6.0, with protease inhibitors at 4°C for 36 hours. Cesium chloride was added to give a density of 1.64–1.69 g/ml, and isopycnic cesium chloride density gradient centrifugation at 40,000 rpm for 48 hours at 20°C with a Spinco SW 50.1 rotor (Hascall and Kimura, 1982) was done. After centrifugation, fractions were collected and dialyzed against distilled water before analysis for protein and uronic acids by the

carbazole procedure (Blumenkrantz and Asboe-Hansen, 1973).

Sulfated glycosaminoglycans were extracted from rockfish muscle by using the methods described by Dietrich et al. (1983) and Jeronimo et al. (1989). These proteoglycans were analyzed on SDS-PAGE.

Electrophoretic Detection of Glycosaminoglycans. Sulfated glycosaminoglycans were separated on SDS-PAGE and stained with either Alcian blue 8GX for carboxyl or sulfate groups (Cook, 1977; Sheehan and Hrapchak, 1980), toluidine blue O for sulfate groups (Nader and Dietrich, 1977), or periodic acid-Schiff reagent for carbohydrate. Sulfated glycosaminoglycan fractions isolated from ice-stored rockfish muscle (up to 7 days) were analyzed on SDS-PAGE for evidence of degradation.

Isolation and Assay of Enzymes. A collagenaselike enzyme was extracted from rockfish skin and muscle tissue by using the procedure of Weeks et al. (1976). The principle of this method is that bound collagenase is dissociated from collagen fibers in the presence of 0.10 M Ca^{2+}. Activity was recovered from rockfish muscle either by heating isolated collagen for 10–15 min at 55°C to 65°C or by incubating it 24 hours at 37°C in the presence of calcium. The activity was recovered in the 30–60% ammonium sulfate fraction.

Metalloproteases were isolated from acetone powder of rockfish muscle, prepared with chilled acetone and butanol as described by Eisen et al. (1973). Approximately 10 g of acetone powder was homogenized (1 min, low speed, Waring Blender) with 200 ml Tris-Cl buffer (0.025 M, pH 7.5) containing 10 mM $CaCl_2$, 0.05% Brij 35, and 0.05% NaN_3. The homogenate was incubated with shaking at 37°C for 1 hour, and then centrifuged at 20,000 x g for 30 min. The supernatant was saved, and the pellet was resuspended in 200 ml of buffer and the procedure repeated. The supernatants were combined and held at 4°C for 48 hours, after which the precipitate formed was removed by centrifugation. The resulting supernatant was brought to 90% saturation with $(NH_4)_2SO_4$ and stored overnight at 4°C. The precipitate was collected by centrifugation and suspended in 50 ml of extraction buffer. The suspension was stirred for 2 hours at 4°C, and the supernatant was collected by centrifugation. Gel filtration of this supernatant was done with a 1.5 x 115 cm column packed with Bio-Gel A-0.5 m equilibrated with extraction buffer containing 0.2 M NaCl. Two peaks with collagenolytic activity were obtained. The active fractions were pooled, dialyzed against water, and lyophilized.

Collagenase substrate was prepared by radiolabeling pure lingcod skin collagen with pyridoxal phosphate and NaB^3H_4 as described by Birkedal-Hansen and Dano (1981). The radiolabeled substrate contained about 2.5 μCi of activity per milligram of collagen. The substrate had an optimum temperature for fibril formation of 15°C, and melting of fibrils occurred at about 20°C and higher temperatures. The assay used (Birkedal-Hansen and Dano, 1981) is based on solubilization of gelled-radiolabeled collagen. Collagenaselike enzymes were also assayed by using substrate SDS-PAGE (Herron et al., 1986; Fields et al., 1990) with 0.1% gelatin, 0.1% lingcod skin collagen, or 0.1% β-casein in polyacrylamide gel. After electrophoresis, gels were incubated for 24 hours at 20°C to 24°C in Tris-Cl, pH 8.0, normally containing 2 mM $CaCl_2$ and 1 mM NEM N-ethylmaleimide. After the gels were stained with Coomassie blue and destained, clear (unstained) zones indicated enzyme activity. The enzyme fraction was also assayed with a synthetic substrate for collagenase (Wunsch and Heidrich, 1963).

B-glucuronidase was isolated and assayed as described by Dutson and Lawrie (1974), and β-N-acetylhexosaminidase was extracted and assayed as described by Stirling (1984). According to Davidson (1970) and Dorfman et al. (1972), these enzymes are involved in the degradation of proteoglycan.

Results and Discussion

Raw Muscle Texture. The texture of raw muscle (kg/20 g) of rockfish after rigor, (24 hours) varied greatly according to the species: yellow eye, 38.8 ± 6.1; starry, 30.5 ± 0.7; canary, 29.9 ± 2.1; bocaccio, 27.2 ± 3.7; yellowtail, 25.1 ± 1.3; and widow, 25.7 ± 1.8 (Chou, 1989). Within species, larger fish had a firmer texture than smaller specimens did. This relationship between fish length and muscle texture was shown for several species, including bocaccio, yellowtail, canary, and brown rockfish (Chou, 1989). The texture of muscle myotomes (separated from myocommata) was also firmer for large fish than for younger year classes of the same species (Chou, 1989). Moreover, rockfish that were live-bled and packed in ice until the resolution of rigor had a consistently firmer texture than specimens that were packed in ice without bleeding.

Softening of Raw Muscle Texture. The softening of muscle from several species of rockfish held on ice for up to 7 days was monitored during ice storage (Chou, 1989). Yellowtail muscle softened much more than bocaccio muscle did (Chou, 1989). The rate of muscle softening was the same for live bled and unbled specimens of the same species (Chou, 1989). Cold sterilization of rockfish (18–24 hours after harvest) with 20,000 or 40,000 KGy ionizing radiation did not influence the rate of softening over 7 days of storage on ice (Chou, 1989), indicating that catabolic reactions by spoilage bacteria do not cause softening of the flesh. However, prolonged storage of sterile rockfish on ice for up to 120 days resulted in development of tough, fibrous muscle similar to that commonly observed in frozen fish (Lim and Haard, 1985). Although the muscle became fibrous, partial loss of fillet integrity occurred at the myocommata-fiber junctions (gaping), indicating breakdown of the extracellular matrix (Chou, 1989).

Storage of sterile rockfish muscle at 30°C resulted in rapid and complete breakdown of the extracellular matrix and in loss of tissue integrity. However, the fibers of this tissue became tough and intractable, and measurement of the

texture of the disintegrated flesh by Kramer shear force indicated toughening rather than softening. We were not able to develop a satisfactory method to measure the loss of tensile strength or of integrity of the muscle tissue. It appears that long-term storage at 0°C or short-term storage at 30°C results in degradation of the extracellular matrix (see following) and cross-linking of the myofibrillar proteins, perhaps because of the formation of dimethylamine and formaldehyde from trimethylamine oxide via the trimethylamine oxide demethylase reaction (Haard, 1990).

Collagen Content of Rockfish Muscle. Based on the hydroxyproline content of muscle from rockfish (bocaccio, yellowtail, chilipepper, greenspotted), the collagen content is about 0.4 g/100 g of wet muscle or 2 g/100 g of muscle dry matter (Chou, 1989). According to Sato et al. (1986), species that have a collagen content of less than 0.5 g/100 g of wet muscle (e.g., sardine, argentine, horse mackerel, brook masu salmon) have a relatively soft raw texture. The collagen content of rockfish muscle was not influenced by the size or year class of rockfish (Chou, 1989). These data indicate that the variable muscle texture of rockfish species cannot be explained on the basis of total collagen content. Hatae et al. (1985) found no relationship between the texture of raw muscle and the gross content of collagen in five species of fish. To the contrary, Sato et al. (1986) reported a positive relationship between collagen content and raw muscle texture of 26 fish species.

Soluble Collagen Content of Rockfish Muscle. Within a given species, the soluble collagen content of muscle 24 hours after harvest was inversely related to the size of the fish. For example, 46-cm bocaccio contained about 45% soluble collagen, whereas only about 25% of collagen was soluble in 68-cm fish (Chou, 1989). Therefore, within a species, soluble collagen content was inversely related to the texture of raw muscle. Possibly, fish muscle collagen, like that of mammals (Eicorn and Butzow, 1966), becomes more highly cross-linked in older animals. Love et al. (1976) suggested that the muscle collagen of Atlantic cod has a higher turnover rate than mammalian collagen does and that the fish collagen does not become progressively cross-linked with age. Little information is available on the cross-link composition of fish collagen. Therefore, we conducted experiments to determine the cross-link composition of rockfish muscle collagen.

Cross-link Composition of Rockfish Muscle. We determined the difunctional cross-link composition of muscle collagen from two rockfish species (Bracho and Haard, 1990). Per mole of collagen, brown rockfish contained 0.24, 0.34, and 0.15 mol of dihydroxylysinonorleucine (DHLNL), hydroxylysinonorleucine (HLNL), and lysinonorleucine (LNL), respectively. In chilipepper rockfish, the main cross-link was LNL (0.49 mol/mol of collagen); the levels of DHLNL and HLNL were 0.19 and 0.14 mol/mol, respectively. These molecular cross-link compositions were quite distinct from the composition of rat tail tendon collagen, which characteristically contains HLNL at about 1 mol/mol of collagen and a much smaller amount of LNL (Eyre, 1987; Reiser and Last, 1986). As the amount of soluble collagen in rockfish muscle is inversely related to fish size, the decrease in collagen solubility may be caused by cross-linking (Snowden et al., 1982).

Change in Soluble Collagen in Iced Rockfish Muscle. Storage of rockfish on ice for 7 days resulted in a progressive increase in soluble collagen. The amount of this increase varied with the species: yellowtail > greenspotted > bocaccio (Chou, 1989) and yellowtail = brown > blue rockfish (Kim, 1990). Within a species, the rate of increase in soluble collagen was not influenced by live-bleeding the fish before storage on ice (Chou, 1989; Kim, 1990). Yellowtail muscle softened extensively, and this was associated with a dramatic increase in the percentage of soluble collagen. In comparison, iced bocaccio muscle showed much less softening and no clear increase in soluble collagen. These data, like those for texture and soluble collagen of different sizes of rockfish 24 hours after harvest, indicate a positive relationship between the amount of soluble collagen and the soft texture of muscle.

Immunodetection of Collagen and Its Degradation Products. A monoclonal antibody prepared from calf skin type I collagen cross-reacted with muscle collagen from several species of rockfish, including yellowtail, brown, chilipepper, greenspotted, canary, blue, widow, and bocaccio. The antibody also cross-reacted with skin collagen from lingcod and several rockfish species. Western blots showed that the antibody reacted equally with α-, β- and γ-chains of collagen and also with types I, III, VI, VII, IX, and X mammalian collagen purchased from Sigma Chemical Co.

It was of interest to determine the ability of this antibody to cross-react with hydrolysis products of collagen chains. Purified α- and β-chains of blue rockfish muscle collagen were hydrolyzed with trypsin, *Staphylococcus aureus* protease, or CNBr. All hydrolysis products 7000 daltons or greater in size reacted with the antibody on Western blots. Further studies were carried out with lingcod skin collagen treated with trypsin, CNBr, or collagenase from *Clostridium histolyticum*. The smallest collagen fragments from lingcod collagen that reacted with antibody on Western blots were 10,000–13,000 daltons. These data indicate that the epitope of the monoclonal antibody is a fairly large sequence of the α-chain common to most if not all collagen types. This appears to be the extended triple-helical regions of collagen chains, which consist of long, unbroken sequences of the repeating tripeptide Gly-X-Y, where X and Y are often proline and hydroxyproline but can be any other amino acid except tryptophan (Bailey and Light, 1989).

SDS-PAGE and Western Blots of Soluble Collagen. Storage of yellowtail rockfish muscle for 1 week results in a progressive increase in the percentage of soluble collagen. SDS-PAGE of this soluble collagen

revealed metachromic bands corresponding in molecular weight to α-, β-, γ-, and higher molecular weight chains. On Western blots of soluble collagen, these bands reacted with antibody, confirming that the bands are collagen chains. The soluble collagen fraction from sterile or nonsterile rockfish muscle that had been held on ice up to 1 week did not show changes in the ratio or amount of α-, β- and γ-chains or formation of metachromic or immunoreactive degradation products having a molecular weight lower than that of the α-chain. Moreover, soluble collagen from sterile muscle held on ice for up to 120 days showed no evidence of collagen degradation (Chou, 1989; Cepeda et al., 1990). However, storage of sterile rockfish muscle for only a few days at 30°C resulted in complete disappearance of metachromic and antibody-reactive bands in the soluble-collagen fraction (Cepeda et al., 1990). Collagen in these samples appears to be degraded to peptides of molecular weight less than about 10,000 daltons. It is known that true collagenases, which act on the collagen fibril, hydrolyze at a specific locus three quarters the length of the molecule from the amino terminal end to yield two triple-helical fragments. We expected that these fragments would be visualized on Western blots. However, the fragments of collagen are unstable and become vulnerable to cleavage by a variety of other proteases in the extracellular matrix (Bailey and Light, 1989).

SDS-PAGE and Western Blots of Total Muscle Collagen. Sterile rockfish muscle was held on ice for up to 14 days, and the muscle protein was made soluble with 8 M urea containing 1% SDS and 50 mM β-mercaptoethanol. SDS-PAGE and Western blots showed no change in the α-, β-, and γ-chains of collagen, and no hydrolytic products of collagen were detected by the antibody (Cepeda et al, 1990). Holding sterile muscle at 30°C for several days resulted in complete disappearance of collagen. These results were like those for the soluble-collagen fraction from iced fish. Experiments were designed to determine if brief (1–6 hours) exposure of flesh to 30°C leads to subsequent collagen degradation during iced storage. The results of such experiments with blue, olive, and widow rockfish were inconsistent. However, when sterile flesh from widow rockfish was held at 30°C for only a few hours, continued degradation of α-, β-, and γ-chains occurred during later storage on ice.

Proteoglycans in Rockfish Muscle. Although proteoglycans are a minor part of intramuscular connective tissue, they appear to play an important role in the retention of water in muscle tissue and may play a role in stabilizing collagen. Dutson (1974) and Dutson and Lawrie (1974) suggested that the increased solubility of collagen in aged beef is caused by the degradation of noncollagen connective tissue components by lysosomal enzymes. We observed that the softening of iced rockfish muscle is associated with an increase in collagen solubility and not hydrolysis of collagen. For these reasons, Sea Grant trainee K. S. Kim focused his research on the proteoglycan fraction of rockfish muscle. The proteoglycan fraction from yellowtail rockfish muscle was isolated by using guanidine hydrochloride extraction and density gradient centrifugation. The proteoglycan fraction recovered contained 30–43 μg of hexuronic acid per 100 g of wet tissue. In contrast, rabbit muscle contains 2200 μg per 100 g of tissue (Parthasarathy and Tanzer, 1987), and bovine nasal cartilage contains 27.5 mg per 100 g of wet tissue (Sajdera and Hascall, 1969).

Electrophoresis of proteoglycans isolated from rockfish muscle 24 hours after harvest revealed several relatively low molecular weight components (74–240 kd) that stained positively for carbohydrate, anionic groups, and specific sulfate residues. The precise role of small proteoglycans is not well understood, but they bind to collagen (Bailey and Light, 1989). Preliminary experiments have shown that the proteoglycans isolated from muscle held on ice for up to 7 days show a progressive decrease in molecular weight (Kim, 1990). If these observations are confirmed by additional experiments, they may help explain the increased solubility of muscle collagen and associated softening of texture during iced storage of rockfish.

Enzymes Involved in Proteoglycan Degradation. The breakdown of proteoglycans has been studied mostly in relation to cartilage degradation (Bailey and Light, 1989). Neutral proteases have been identified that act on the protein core. The glycosaminoglycan side chains can be hydrolyzed by glycosidases like β-N-acetylhexosaminidase. The sulfate groups may be removed by sulfatases and the hexoses by iduronidases and glucuronidases. In this project, β-glucuronidase and β-N-acetyl hexosaminidase were detected in yellowtail rockfish muscle (Kim, 1990). The activity of enzymes isolated from yellowtail muscle held on ice for up to 7 days did not change.

Enzymes Involved in Collagen Degradation. The collagen triple helix is highly resistant to the action of most proteases, but cells in the connective tissue synthesize and secrete a group of proteases that act on collagen. The best characterized of these proteases is mammalian collagenase, a Zn^{2+}-metalloproteinase. Degradation of collagen *in situ* involves the concerted action of a complex series of several enzymes (Bailey and Light, 1989).

An enzyme fraction that hydrolyzed native collagen fibrils at neutral pH was isolated from rockfish skeletal muscle. The enzyme fraction was separated into two active components on collagen and gelatin zymograms. The enzymes were not active in casein zymograms; casein in solution; or synthetic substrates for trypsin, chymotrypsin, and collagenase. According to the results of SDS-PAGE and gel permeation chromatography, the proteases had molecular weights of 98,000 and 47,000. Both enzymes had an optimum pH around pH 9 and were heat stable. The larger protease was stable for 1 hour at 60°C in buffer, pH 8.0, containing 2 mM $CaCl_2$, and 1

mM NEM. The smaller enzyme was inactivated after 1 hour at 60°C but was stable after 1 hour at 50°C in the same buffer. The rockfish enzymes were activated by Ca^{2+} (2 mM), NEM (1 mM), and p-APMA 4-aminophenyl mercuric acetate (1 mM) and completely inhibited by 10 mM EDTA or 10 mM 1,10-phenanthroline. The activity of these enzymes was not influenced by 1 mM phenylmethyl-sulfonyl fluoride, 1 mM DTT dithiothreitol, or 0.5% SDS. Thus, both enzymes can be classified as heat-stable, alkaline metalloproteinases. The activity of these enzymes in SDS-PAGE substrate (collagen or gelatin) gels depended on electrophoretic separation before assay. Thus, it is possible that metalloproteinase inhibitors are present in the enzyme fraction. Electrophoresis would separate inhibitor(s) from the metalloproteinases, making them free to act on the collagen or gelatin in the polyacrylamide gel. Experiments are under way to obtain homogeneous preparations of both metalloproteinases. These enzymes may be activated in temperature-abused fish, perhaps by *in situ* degradation of inhibitor(s).

Conclusions

The texture of rockfish flesh varies with the species of *Sebastes* and is more firm in large fish than in small fish. The collagen content of flesh is not influenced by the species or size of rockfish. Larger rockfish have less soluble muscle collagen than smaller rockfish of the same species. Distinctive difunctional cross-links are present in rockfish muscle collagen and may explain the firmer texture of muscle from older animals.

The flesh of Pacific rockfish softens during storage on ice after harvest. The rate of softening varies with the species and is not influenced by spoilage bacteria or live bleeding. The solubility of muscle collagen increases in iced fish and correlates positively with softening of the flesh. Collagen chains are not degraded; however, the proteoglycans undergo a decrease in molecular size in iced fish.

The connective tissue matrix of rockfish flesh is completely destroyed, and collagen chains are degraded during postharvest storage at 30°C. These changes are caused by endogenous factors and are not caused by bacterial spoilage. A few hours exposure of flesh to 30°C can begin limited degradation of collagen that will continue during subsequent ice storage.

Enzymes that can degrade proteoglycans and collagen are present in rockfish muscle. Collagenolytic enzymes detected in rockfish muscle are heat stable, alkaline metalloproteinases. Inhibitor(s) that prevent the catalytic activity of these collagenases in tissue extracts are removed by electrophoresis in polyacrylamide gels containing the detergent SDS.

Cooperating Organizations

National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Tiburon, California
Starkist Foods, California
University of Massachusetts Marine Station

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Pre-exploitation Abundances of Important Large Recreational and Commercial Fishes off Southern California

University of California, San Diego
R/F-125
1988-1990
Project Initiated: October 1, 1988
Project Completed: January 31, 1990

Paul K. Dayton and Alec D. MacCall

Introduction

The goal of this Sea Grant project was to estimate pre-exploitation abundances for the white seabass (*Atractoscion nobilis*), the yellowtail (*Seriola lalandei*), and the giant sea bass (*Stereolepis gigas*). Baseline measures of abundance or biomass of fish and wildlife populations are valuable for assessment of human-induced changes in those populations. Such measures are, however, rare. Populations may be altered long before the need for baseline information is recognized.

Commercial fisheries for the white seabass, yellowtail, and giant sea bass were well established by the late 1910s and early 1920s, a period in which significant changes occurred in the populations of these three large predatory fishes (Figures 1–3). Fisheries data from this period to 1990 suggest that California populations of these fishes are in various states of depletion, but prefishery population levels have not been estimated.

A unique data set collected by the Avalon Tuna Club of Santa Catalina Island (Figure 4) contains information that predates large-scale commercial fishing off Southern California (Macrate, 1948). This data set contains records of the heaviest white seabass, yellowtail, and giant sea bass caught each year by a member of the club. To the extent that size composition reflects fishing pressure, these data provide a means of estimating pre-exploitation biomasses for white seabass and yellowtail populations by using a maximum likelihood approach. Estimates are made by combining a simple population model and a statistical model. Our original intent was to use the same modeling approach for the giant sea bass. However, inadequate data on the life history of this fish precluded use of this maximum likelihood approach; a lower limit of its pre-exploitation biomass was estimated by using catch data only.

The General Model

Maximum likelihood estimates of the pre-exploitation abundances for the white seabass and the yellowtail were generated by combining (1) a harvested population model generating the distribution of yearly weight frequencies, (2) a statistical model for the probability distribution of the heaviest fish in a sample from the model population, and (3) annual observations of the heaviest fish caught by a member of the Avalon Tuna Club. The statistical model links the records of the Avalon Tuna Club to the distribution of weight frequencies in the modeled population, providing a basis for estimating the likelihood that the Avalon Tuna Club data would have been observed, given the assumed population model.

The Population Model. The purpose of the population model is to provide distributions of annual weight frequency of an assumed resident, self-sustaining population in California waters. Components of the model include length-at-age and weight-at-length relationships, a stock-recruitment relationship (Figure 5), age of recruitment to the fishery, and the historical commercial and sport harvests. Fitted parameters from the population model are initial population size and instantaneous mortality rate.

By assuming a constant instantaneous natural mortality rate (M) and an initial population size (B_o), we constructed a stable age

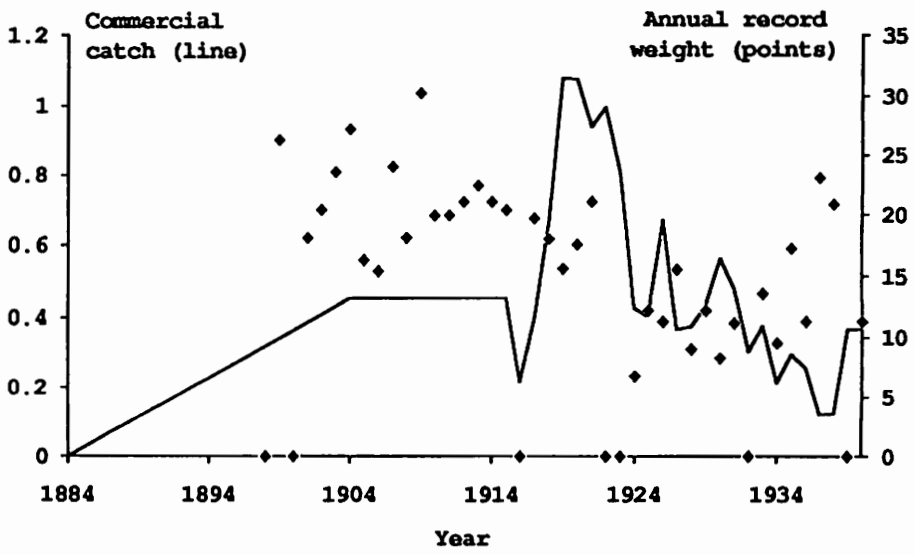

Figure 1. Commercial catch (1000 tons) and record weight (kg) of white seabass caught by a member of the Avalon Tuna Club, 1884–1940. Commercial catch for the period 1904 to 1915 set constant at 454 tons and linearly interpolated to that level beginning with zero catch in 1884 (Frey, 1971). See text for remaining data sources.

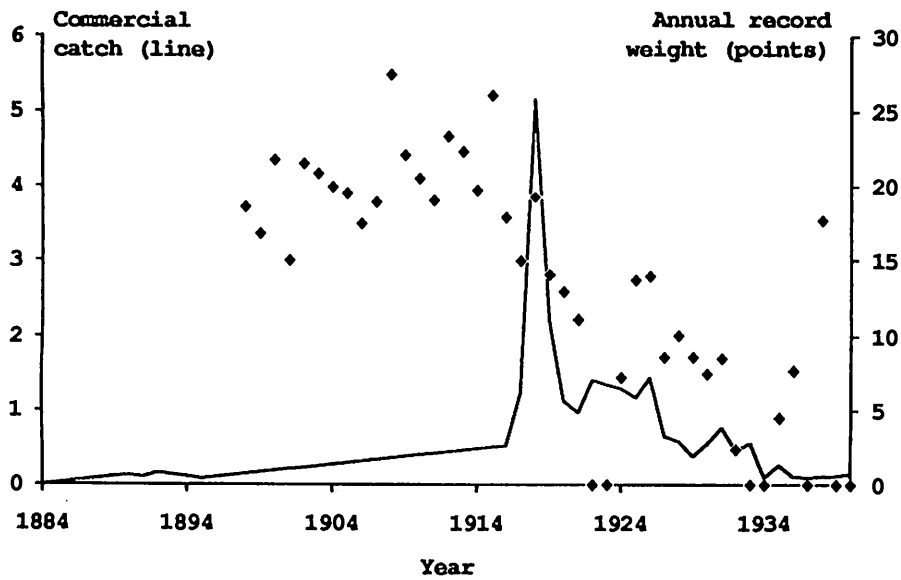

Figure 2. Commercial catch (1000 tons) and record weight (kg) of yellowtail caught by a member of the Avalon Tuna Club, 1884–1940.

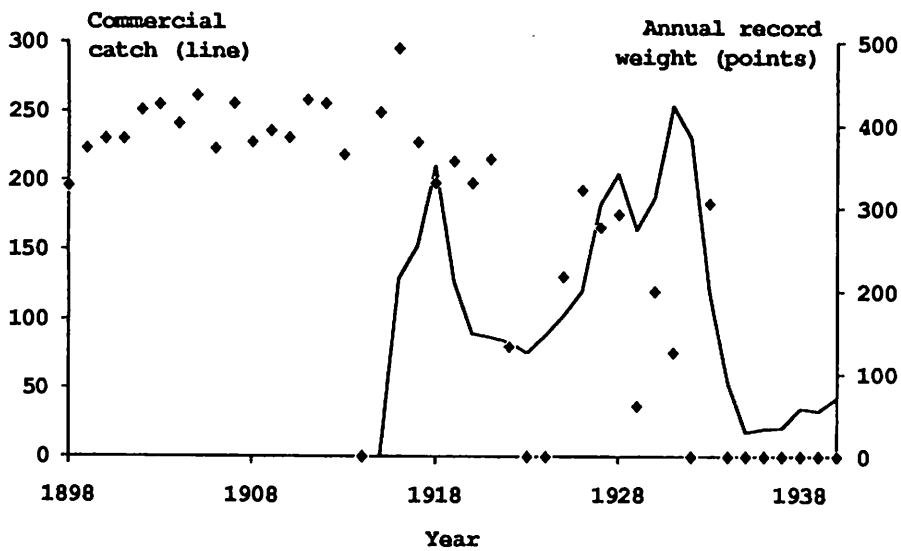

Figure 3. Commercial catch (1000 tons) and record weight (kg) of giant sea bass caught by a member of the Avalon Tuna Club, 1898–1940.

frequency distribution. Age-length (with variability) and length-weight relationships from the literature were used to convert the age frequency distribution to a weight frequency distribution, with fish summed over discrete weight categories of unit width. Constant recruitment to the model population in the absence of other extraneous perturbation leads to a constant population abundance and weight frequency distribution over time.

Such constancy ceases under conditions of harvesting, and the weight distribution subsequently varies with the removal of portions of the model population according to historical commercial and sport catch records.

The population model runs from 1884 to 1940. Avalon Tuna Club records begin in 1898. The period from 1884 to 1897 is included to incorporate the effects of earlier years of commercial fishing for which records are available.

The Statistical Model. Based on a year-specific weight frequency distribution from the population model, the statistical model provides a method of determining a year-specific probability distribution for the heaviest fish in a sample from the population. This extreme-value probability distribution is taken from Hogg and Craig (1965):

$$f(W_{max} \mid S) = S\,[F(W)]^{S-1} f(W), \qquad (1)$$

where S, $f(W)$, and $F(W)$ are the sample size, weight frequency distribution, and cumulative weight frequency distribution, respectively, for the model population. Because the year-specific weight frequency and cumulative weight frequency distributions ($f(W)$ and $F(W)$, respectively) are functions of the population model and its estimated parameters, the resulting year-specific probability distribution for the heaviest fish taken in a sample from the population is also a function of those parameters. Although the Avalon Tuna Club records do not include sample size, and hence true sample size is unknown, the mean sample size can be assumed to be approximately proportional to the year-specific abundance in numbers of fish (N) given by the population model. The constant of proportionality (q, where $S_{mean}=qN$) is a parameter to be estimated. Because actual S varies about S_{mean}, and is a relatively small number, S is assumed to have a Poisson distribution. The density function used in the likelihood equation is the weighted mean of the $f(W_{max} \mid S)$ values from equation (1), with weighting from the Poisson frequency distribution $p(s)$. Thus, if a maximum weight observation exists for a particular year,

$$f(W_{max} \mid S_{mean}) = \sum_{n=1}^{\infty} [f(W_{max} \mid s) p(s \mid S_{mean})], \qquad (2a)$$

and if there is no observation,

$$f(no\ obs) = p(s = 0 \mid S_{mean}). \quad (2b)$$

Note that lack of a recorded maximum-sized fish for a particular year (which occurs in these time series) is likely to indicate that recreational catch rates, and hence available biomass, were low. The stochastic treatment allows this inference to be incorporated explicitly in the likelihood function.

By comparing the observed annual records of the Avalon Tuna Club with the corresponding probability distribution for the heaviest fish, a year-specific probability can be determined for each year of the interval 1898 to 1940 in the model simulation. The sum of the natural logarithms of those year-specific probabilities over the period 1898 to 1940 is the log-likelihood function for the population model and its specific input.

The simplex algorithm (Nelder and Mead, 1965) is combined with a single- and double-parameter searching method to locate the combination of parameters that leads to the maximum likelihood. Those parameters include the pre-exploitation abundance and the instantaneous mortality rate from the population model and the constant of proportionality for sample size from the statistical model.

Two methods of testing precision of the results were used. The first estimated minimum-variance bounds for the parameters by using second and mixed partial derivatives (Norden, 1972, 1973). The second method varied the assumptions of the population model, including the stock-recruitment function, the age of recruitment to the fishery, the biomass of seasonal migrants to the population and commercial fishery, the period of extensive sport fishing, the variability and density dependence of length-age functions, and the stability of the starting age distribution.

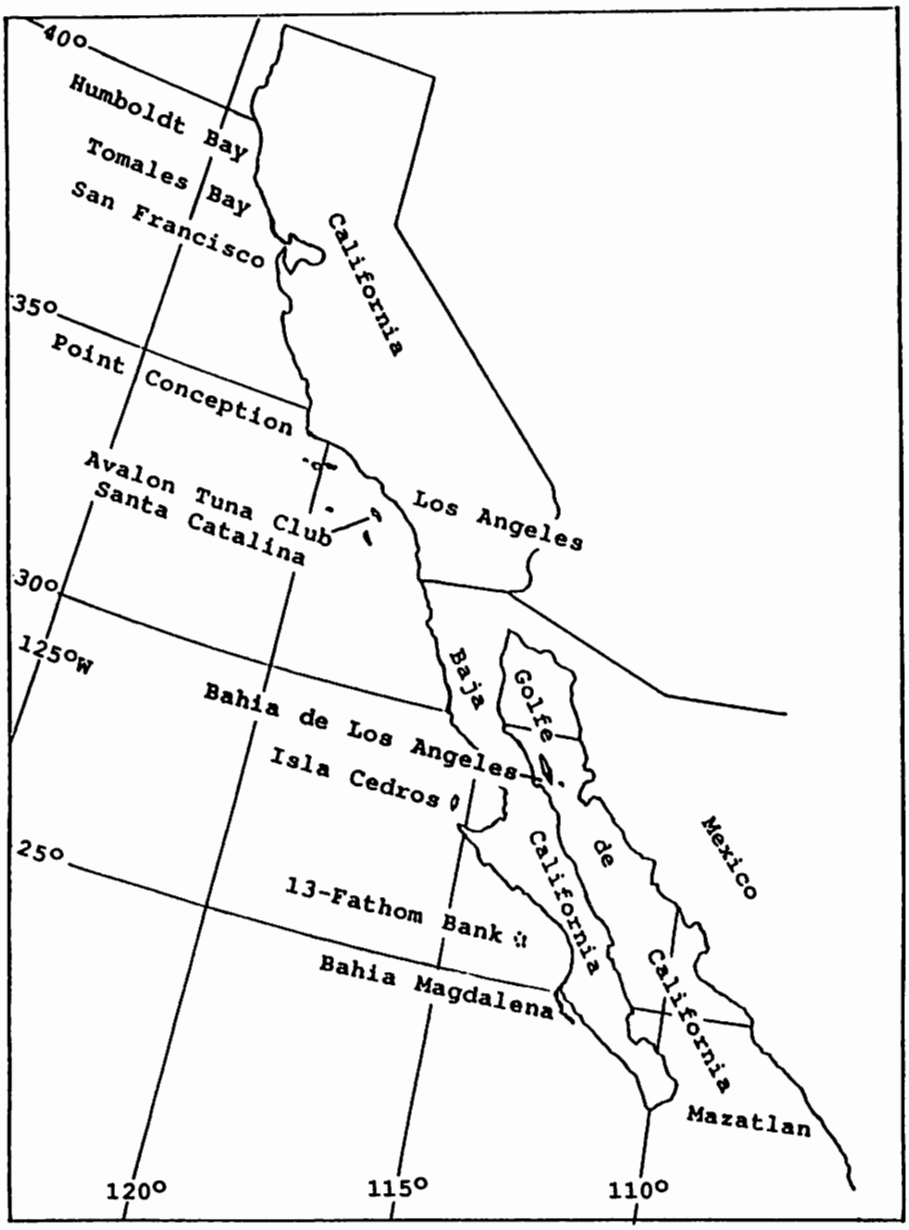

Figure 4. Oceanic area pertinent to study.

Results

White Seabass. Model simulations suggested that the pre-exploitation biomass of white seabass was near or slightly greater than 20,000 tons, with coefficients of variation on the order of 0.25 to 0.4. Corresponding population abundances ranged from approximately 2 million to 2.5 million fish. Results also suggested an instantaneous natural mortality rate on the order of 0.08 yr^{-1} (coefficient of variation ~ 0.15), somewhat lower than previous estimates of 0.33 yr^{-1} (Thomas, 1968) and 0.12 yr^{-1} (MacCall, et al., 1976). Figure 6 shows the correspondence of model biomass to annual record weight of white seabass taken by the Avalon Tuna Club. Similarly, the correspondence of model biomass to annual commercial catch is shown in Figure 7.

Yellowtail. Estimates of pre-exploitation biomass and abundance for the yellowtail were similar to the estimates for the white seabass, that is, just greater than 20,000 tons and 2 million fish, respectively. However, the coefficients of variation for the

yellowtail were on the order of 0.05 to 0.12, and therefore were much smaller than is the case for the white seabass. Instantaneous natural mortality for the yellowtail was estimated to be about 0.09 yr^{-1}, slightly greater than that of the white seabass. The correspondence of model biomass to annual record weight of yellowtail taken by the Avalon Tuna Club is shown in Figure 8; Figure 9 shows the correspondence of model biomass to annual commercial catch.

Giant Sea Bass. As noted before, insufficient information on the life history of the giant sea bass, particularly age-length-weight relationships, precluded the estimation of pre-exploitation biomass for this fish with the model used here. However, Frey (1971) concluded that the combination of commercial fishing, slow recruitment, and continued sportfishing has inhibited the recovery of the giant sea bass. If this is the case, then the lower limit for that biomass can be approximated by summing the total commercial catch of giant sea bass during the period from 1916 (when records begin for the commercial fishery) to 1940. The sum of those landings is approximately 1300 tons. Certainly, this estimate could be improved if information on growth and mortality of the giant sea bass and estimates of the sportcatch during this period were available. Still, the sportcatch before 1940 probably was negligible relative to the commercial catch, and regardless of growth and mortality, recruitment is sufficiently low to prevent rapid turnover of the population.

Discussion

The essence of the maximum likelihood approach used here is the search of a multivariate response surface for its peak. In this model, the likelihood surface was a function of three parameters that were subject to at least one major constraint: The initial abundance and mortality rate must allow an initial population sufficiently large to withstand the removal of the biomass taken in the commercial catch. On the response surface, this constraint manifests itself in the form of a boundary beyond which any solution is infeasible. Figure 10 illustrates likelihood response surfaces for the white seabass and the yellowtail when only mortality and initial abundance are varied. For both the white seabass and the yellowtail, the peak of the surface is located close to this boundary of feasible solutions, suggesting that these populations were fished to low levels during the early part of this century. The close correspondence between the parameter estimates for the white seabass and the yellowtail suggests these two fishes were similar in population size and have similar life histories.

In general, results for the white seabass and the yellowtail were robust to the multiple assumptions required by this modeling approach. Furthermore, these results are the only available estimates of pre-

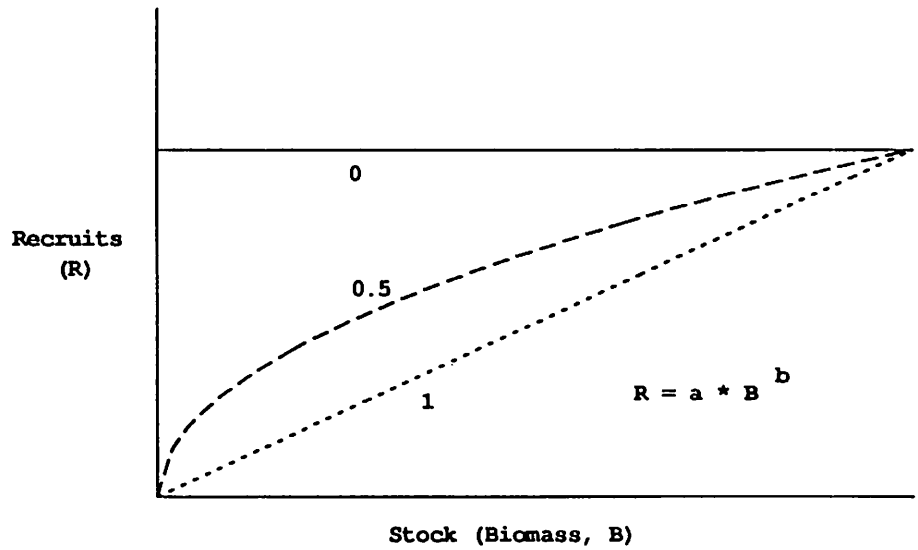

Figure 5. Stock-recruitment relationships used in the model. The three lines correspond to the indicated values of b.

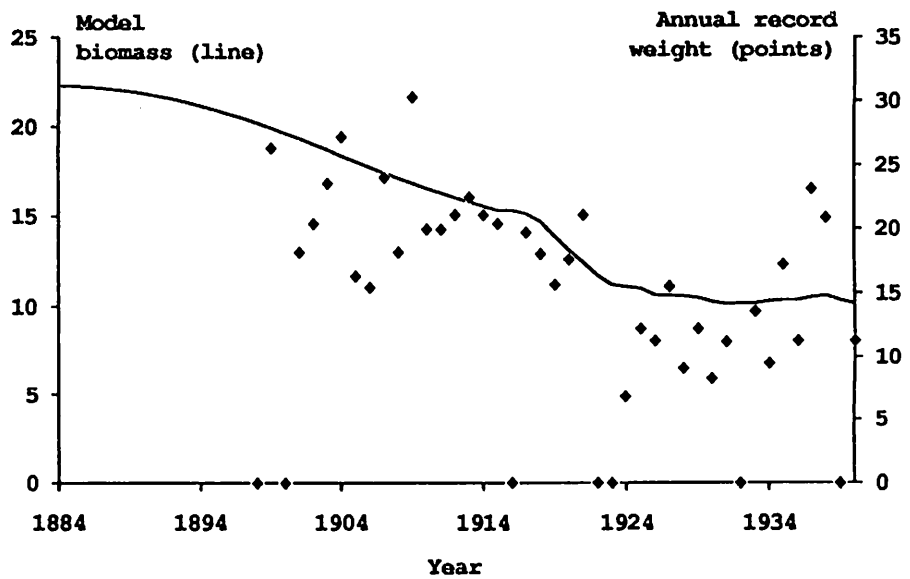

Figure 6. Model biomass (1000 tons) and annual record catch (kg) of white seabass by an Avalon Tuna Club member, 1884–1940.

exploitation abundance for the white seabass and yellowtail. As such, they provide the only indication of the severity of historical exploitation and the extent of recovery that would be necessary to return these populations to their natural state. Clearly, however, these estimates apply to the natural state in the late 1800s. To suggest that in the absence of exploitation these fish populations would be at similar levels today requires the assumption that the overall influence of the pertinent demographic and environmental conditions has not changed. In the absence of information on such changes, the estimates generated here provide our best indication of the natural state of these fishes.

As such, this information should be useful as a guide for programs such as California's Ocean Resources Enhancement and Hatchery Program (OREHP). One of the projects funded by OREHP has been development of a cost-benefit assessment model of hatchery performance and stock rehabilitation, with particular emphasis on the white seabass. As the population dynamics of the white seabass have heretofore not been well known, the OREHP investigation has necessarily relied on many assumptions about vital rates and related population properties. Although the white seabass is thought to be depleted, data have been insufficient to obtain clear results by using standard fishery assessment models (MacCall et al., 1976).

To the present, assessment and management of the white seabass, the yellowtail, and the giant sea bass have been based on the assumption that California's catches are taken from the seasonal northward migrants, which has largely been the case over most of the last half century. Managers have understandably hesitated to impose restrictions on harvests from a resource over which they would seem to have little real control. Management based on conservation of a strictly resident stock would require much greater restrictions on fishing effort. In addition, our results indicate natural mortality is low, which also suggests fishing mortality must be kept low if a resident stock is to be sustained. Presumably a population composed of both resident and migrant fish would allow intermediate levels of fishing. Until now, lack of sufficient information on the early resources and fisheries has prevented quantitative consideration of resident Southern California stocks of these species. The estimates provided by this study give managers a new view of the potential productivity of these stocks in Southern California. For example, the results allow application of the Gulland potential yield rule-of-thumb, $Y_{pot} = MB_o/2$, where Y_{pot} is potential yield. These cases indicate a Y_{pot} on the order of 500 to 900 metric tons. Indeed, the recent interest in artificial propagation of white seabass would seem to be more consistent with a management of a resident rather than a migrant resource. If artificial

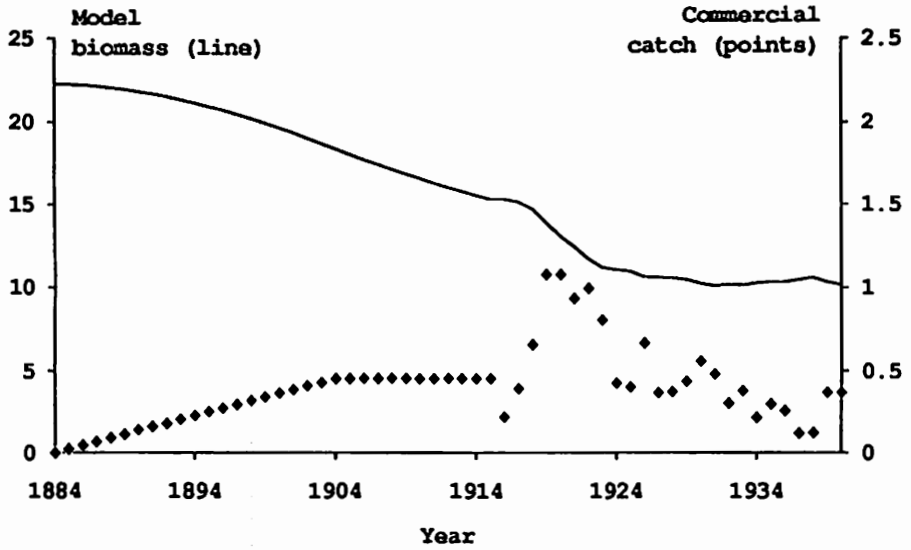

Figure 7. Model biomass and commercial catch of white seabass, 1884–1940. Both vertical axes are in units of 1000 tons.

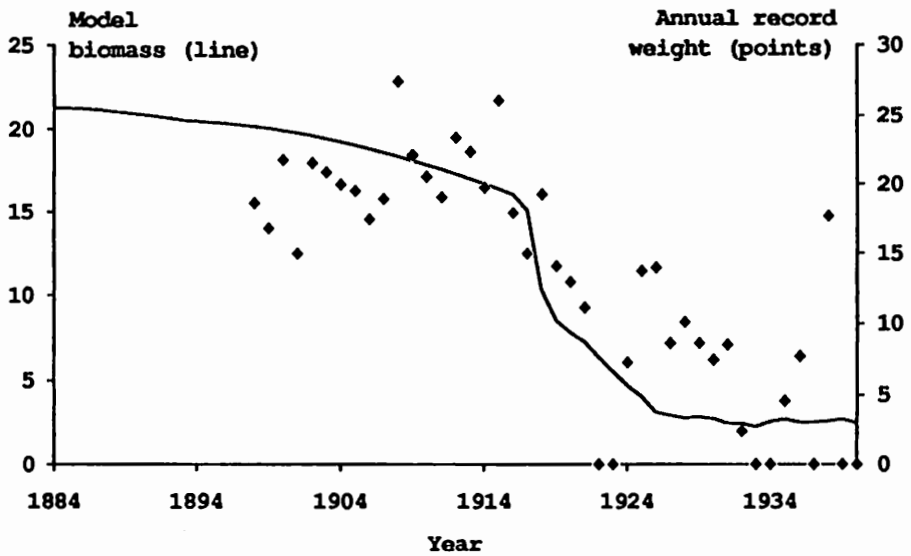

Figure 8. Model biomass (1000 tons) and annual record catch (kg) of yellowtail by an Avalon Tuna Club member, 1884–1940.

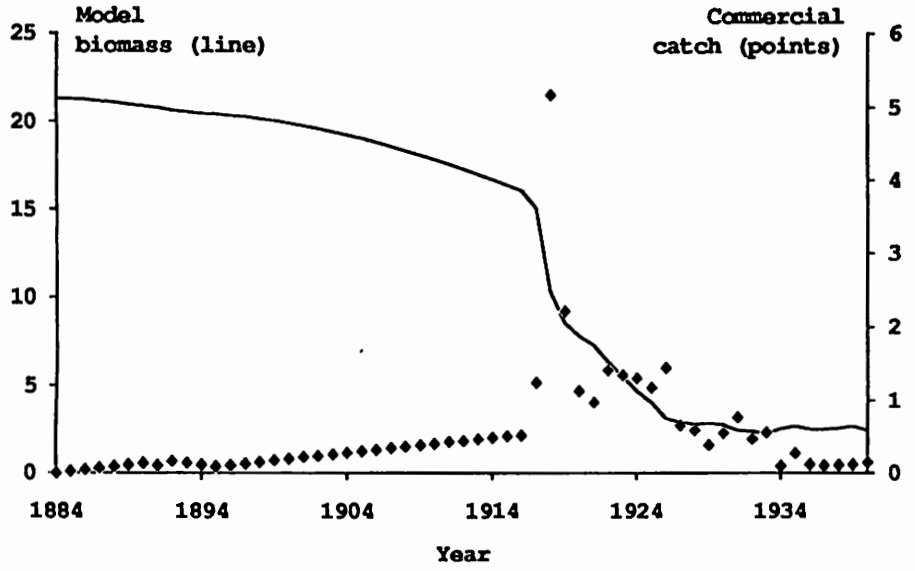

Figure 9. Model biomass and commercial catch of yellowtail, 1884–1940. Both vertical axes are in units of 1000 tons.

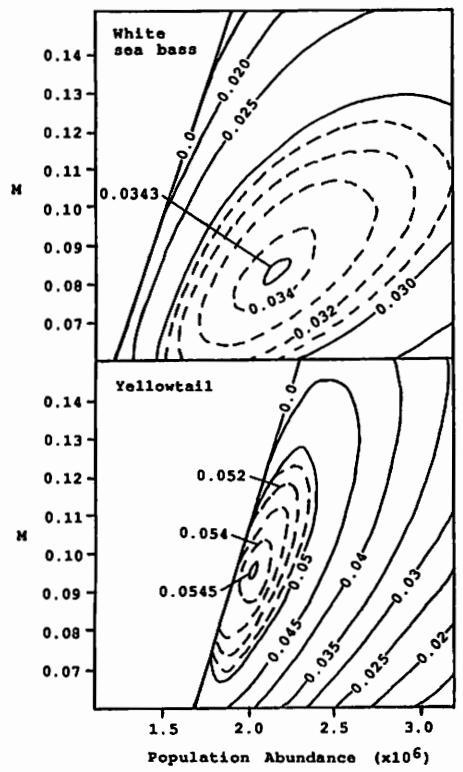

Figure 10. Contour plots of model response surfaces for the white seabass and the yellowtail. Isobars are of geometric mean probabilities, determined as antilog (log-likelihood/43 years).

propagation is to be attempted seriously, it is desirable and perhaps essential that management of these populations in Southern California be made consistent with conservation of a resident population. This study provides information which will help fishery scientists evaluate and managers decide whether the benefits of resident-based population management are worth the restrictions that would have to be imposed on fisheries.

Cooperating Organizations

Avalon Tuna Club, Avalon, California
California Department of Fish and Game, Long Beach, California
National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, California
University of California, San Diego Supercomputer Center

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Ragen, T. J. 1990. Pre-exploitation abundances for the white seabass (*Atractoscion nobilis*), yellowtail (*Seriola lalandei*), and giant sea bass (*Stereolepis gigas*) off Southern California. In The estimation of theoretical population levels for natural populations. Doctoral dissertation, University of California, San Diego.

Lectures

MacCall, A. D. Historical changes in the California Current ecosystem. Seminar presentation. NOAA/NMFS Tiburon Laboratories, Tiburon, California, March 1990.
MacCall, A. D. Historical changes in the California Current ecosystem. Seminar presentation. University of California Bodega Marine Laboratories, Bodega Bay, California, March 1990.
MacCall, A. D. Historical changes in the California Current ecosystem. Seminar presentation. California State University, Hayward, California, May 1990.

Extending Prime-Quality Market Life of Seafoods

University of California, Davis
R/F-127
Project Initiated: October 1, 1988
Project Completed: September 30, 1990

Norman F. Haard and David M. Ogrydziak

The foremost problem in the seafood industry in the United States is the inability to meet the growing demand for high-quality (grade A) products. The industry can meet this demand by adopting techniques that extend the shelf life of fresh fish. The "ice time" for excellent product (grade A) may be only 3 or 4 days; that for edible or mediocre product (grade B) is normally about 15 days. Most documentation of ice time for seafood is based on the grade B standard, the time at which the product is totally unacceptable. This project focused on factors and treatments after harvest that affect grade A quality. A major objective was to determine the factors that determine the grade A shelf life of fish when it is pasteurized by ionizing radiation and/or stored in modified atmosphere.

Methods

Biological Specimens. Rockfish were caught with fishing poles on the Cordell Banks or near Point Reyes in northern California and immediately packed in ice. The fish were transported to the laboratory in Davis, California, where they were filleted, skinned, and packed in bags within 8 hours after harvest. Packaged samples were treated (see the following section) and held on ice for up to 7 days. Most of the research carried out in 1988–1989 was done with blue rockfish (*Sebastes mystinus*) and yellowtail rockfish (*Sebastes flavidus*) ranging in size from 20 to 30 cm. In 1989–1990, most studies were carried out with yellowtail rockfish 30–40 cm in length.

Treatments. In the modified atmosphere (MA) treatment, fillets were packed in Cryovac barrier bags (type B 540, W. R. Grace & Co., Hayward, California) and flushed with a mixture of 80% CO_2 and 20% air (Brown et al., 1980) by using a Hanover/Turbovac vacuum packaging machine (Kansas City, Missouri). The packaging material used has low permeability to oxygen (3–6 cc/m^2, 24 hours, 1 atm, 0% relative humidity). Tests showed that the gas composition of sealed bags remained constant during several weeks storage in air at 0°C. The gas composition of the modified atmosphere was monitored by gas liquid chromatography with a Carle Model 8000 GLC.

For treatment with ionizing radiation, fillets were vacuum packaged in Cryovac barrier bags, packed in ice, and treated with 1 kGy of ionizing radiation at the Cobalt-60 facility at UC Davis. One kiloGray (kGy) of radiation is classified as a pasteurizing dose, which normally decreases total bacterial numbers by about 90% (Licciardello et al., 1984, 1986).

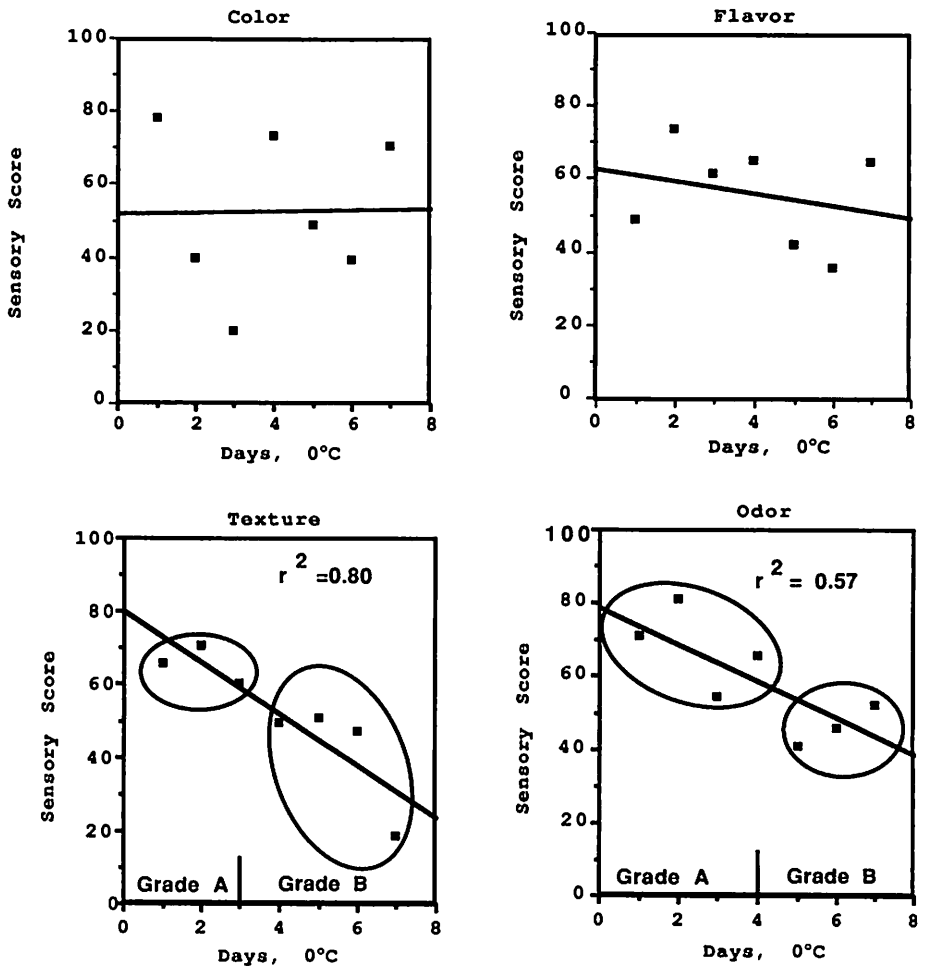


Figure 1. Sensory attributes of rockfish stored on ice for 1–7 days and then cooked. Mean separation (circles) indicates break point in grade A and grade B quality. Data are representative of two storage trials with blue rockfish.

Sensory Evaluation. Individuals were selected for training as expert tasters on the basis of their interest in tasting fish and their ability to discriminate coded samples of fresh (0–4 days ice time after harvest) and less fresh (5–7 days ice time) cooked rockfish by a triangle test. The six panelists who were selected were not otherwise involved with this project. They were trained to evaluate texture, appearance, flavor, and odor of cooked rockfish. Texture, odor, color, and flavor of rockfish held at 0°C for 1–7 days were evaluated daily by the trained panel by using the interval scale method (Stone and Didel, 1985). The results were analyzed by analysis of variance and mean separation with the Student-Newman-Kuels test with SAS software. A descriptive chart was developed for distinguishing grade A and grade B rockfish. Rockfish treated with 1 kGy ionizing radiation, MA, or a combined treatment were held at 0°C for 7 days and evaluated daily for texture, flavor, odor, color, and overall quality to determine the transition time from grade A to B.

Microbial analysis. Total aerobic and anaerobic plate counts were determined by methods based on the U.S. Food and Drug Administration (1984) and Association of Official Analytical Chemists (1984) procedures. The culture medium was plate count agar (standard method agar) containing 0.5% NaCl as recommended for microbial analysis of marine products (Lee and Pfeifer, 1974; Pelroy and Eklund, 1966; Silverrio and Levin, 1967). The diluent for serial dilutions was 0.9% NaCl (Gerhardt et al., 1981).

A fillet to be analyzed was transferred to a sterile cutting board and minced to cubes about 0.5 cm on a side with a flamed knife. A weighed aliquot (normally 25 g) was homogenized with 225 ml of sterile 0.9% NaCl in a Waring Blender for 1 min at high speed (10^1 dilution). The homogenate (1 ml) was diluted with 9 ml 0.9% NaCl (10^2 dilution) and serially diluted with 0.9% NaCl up to 10^5.

Aliquots (1.0 ml) of each dilution were plated in triplicate, and the plates were inverted and incubated

Table 1. Grade A Market Life and Characteristics of Rockfish Fillets Held in Air at 0°C

Attribute	Market Life (Days at 0°C)	Characteristics
Cooked texture	3	Grade A texture is characterized by springy, juicy, cohesive mouth feel. Grade B texture is characterized by mushy, dry, fibrous, and flaky mouth feel.
Cooked odor	3	Grade A odor has neutral or light fish smell, fresh crab meat odor. Grade B has slight sulfur and ammonia odor.
Raw appearance	4	Grade A appearance includes pink to red "red" muscle and translucent "white" muscle. Grade B appearance includes pale red, brown, or green "red" muscle and opaque "white" muscle.
Overall rating	3	

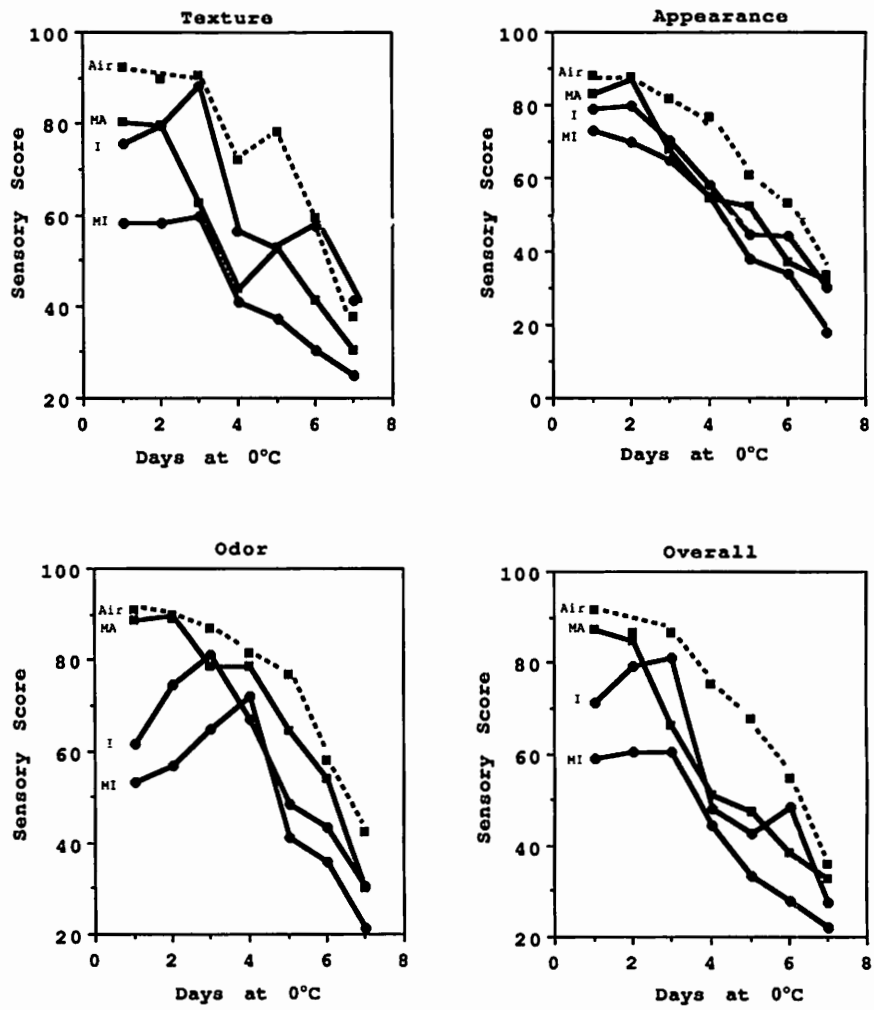


Figure 2. Sensory evaluation of fillets after storage at 0°C. MA = modified atmosphere, I = kGy radiation, MI = kGy radiation and storage in modified atmosphere. Data are average of three experiments with blue and yellowtail rockfish.

48 ± 2 hours at 37°C for aerobic plate counts. For anaerobic counts, plates were placed in an anaerobic environment by using the GasPack jar system (BBL Microbiology Systems, Cokeysville, Maryland) and incubated 5 days at 20°C as described by Gerhardt et al. (1981).

Chemical Analyses. For nucleotide analysis (K value), adenosine triphosphate (ATP), and its degradation products were determined by high performance liquid chromatography with an ^{18}C reverse-phase column by a modification of the procedure of Woyewoda et al., (1986). The procedure was modified by using 0.05 M potassium phosphate, pH 7.0, containing 0.5% methanol as modifier in the eluant buffer at a flow rate of 1.5 ml/min. K value was calculated by using the following equation:

$$\frac{[hypoxanthine(HPX)] + [inosine(IN)]}{[ATP]+[ADP]+[AMP]+[IMP]+[IN]+[HPX]}$$

Solubility of myofibrillar protein, an index of protein denaturation and loss of succulent meat texture, was measured by the method outlined by Lim and Haard (1984). Dimethylamine (DMA), a product of trimethylamine oxide (TMAO), which has been linked to protein denaturation, was measured by the method outlined by Woyewoda et al. (1986). Lipid oxidation was estimated by the thiobarbituric acid (TBA) test as described by Woyewoda et al. (1986).

Lipid hydrolysis was estimated by measuring titratable acidity in a fat extract and was calculated as oleic acid equivalents as a percentage of either total fat or wet tissue weight (Woyewoda et al., 1986). Triplicate samples of both dark and white muscle from separate fillets were evaluated after storage in air or MA for 0, 3, 6, 9, 12, 15, 18, and 21 days. Aliquots (5 ml) of chloroform extract were transferred to test tubes, the headspace of tubes was flushed with nitrogen gas, and the sealed tubes were stored at –70°C for about 4 weeks before analysis for specific free fatty acids by gas liquid chromatography.

Free fatty acids in the oil extracts of fish held in air or MA were

Table 2. Grade A Market Life of Air Control and Treated Rockfish Fillets Based on Sensory Evaluation

	Grade A Storage Life (days at 0°C)			
Attribute	Air Control	MA	I	MA + I
Raw appearance	4	2.5	2.0	1.0
Cooked texture	3	1.0	<1	<1
Cooked odor	3	2.5	<1	<1
Overall acceptance	3	2.0	<1	<1

Note. Data are average of results obtained in three separate storage trials with blue rockfish. Values are rounded to nearest half day. MA = modified atmosphere, I = ionizing irradiation.

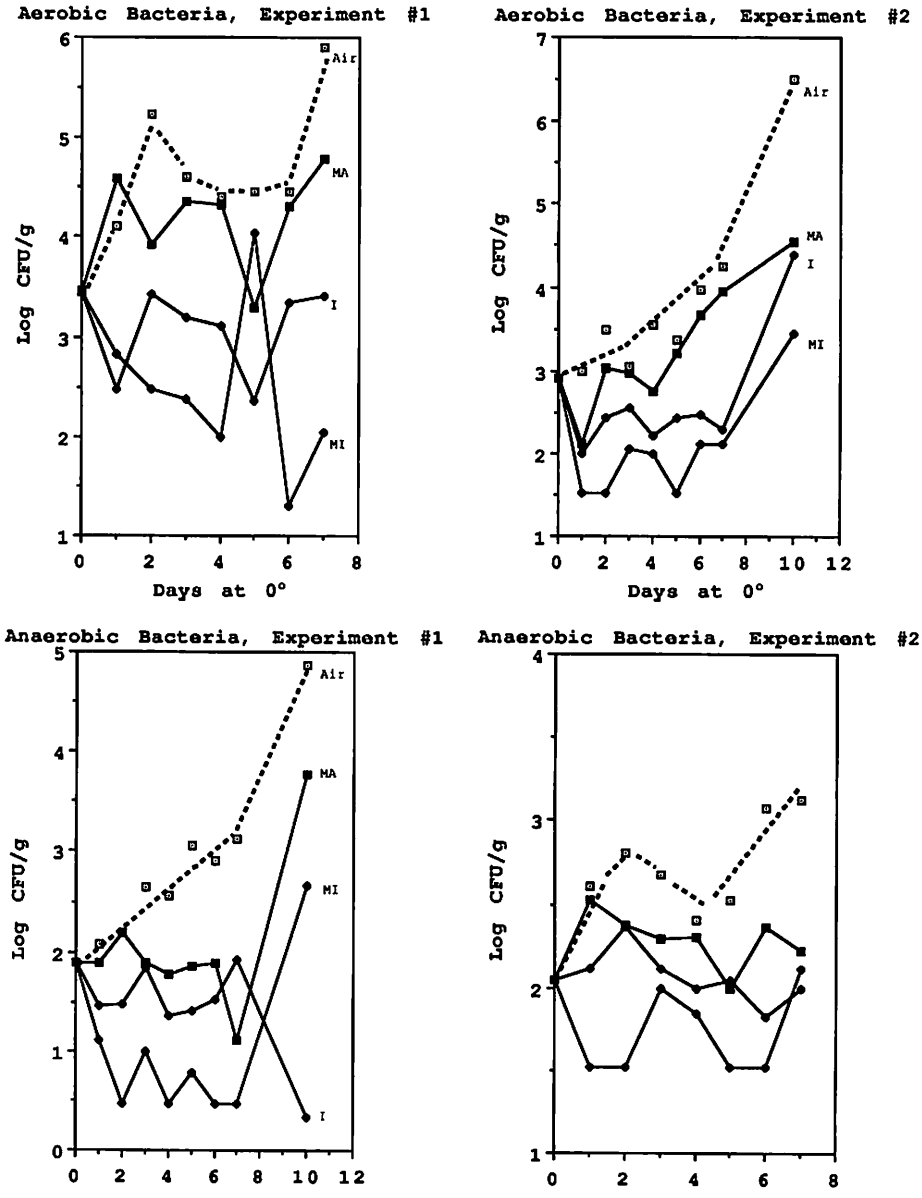


Figure 3. Total plate counts of fillets held at 0°C. Experiment #1 was with blue (aerobic) and olive (anaerobic) rockfish. Experiment #2 was with yellowtail rockfish. CFU = colony-forming units.

Table 3. Bacteria Plate Counts of Rockfish Fillets at the End of Grade A Market Life

Storage or Treatment	Grade A Market Life (Days at 0°C)	Aerobic Count (log CFU/g)	Anaerobic Count (log CFU/g)
Air Control			
Experiment 1	3.0	4.6	3.0
Experiment 2	3.0	3.5	2.7
Modified Atmosphere			
Experiment 1	2.0	3.9	2.2
Experiment 2	2.0	3.0	2.4
Irradiated			
Experiment 1	0.5	3.1	1.8
Experiment 2	0.5	2.5	2.1
Modified Atmosphere/Radiation			
Experiment 1	0.5	2.7	1.4
Experiment 2	0.5	2.2	1.8

Note. Experiment 1 (aerobic) was done with blue rockfish with an initial bacterial population of 3.4 colony-forming units (CFU)/g; experiment 1 (anaerobic) was done with olive rockfish with an initial bacterial population of 2.0 CFU/g; experiment 2 (aerobic) was done with yellowtail rockfish with an initial bacterial population of 3.0 CFU/g; experiment 2 (anaerobic) was done with yellowtail with an initial bacterial population of 2.0 CFU/g.

determined by gas liquid chromatography according to the method of Lepage and Roy (1986). The method was modified by using 0.15 ml of a chloroform extract of fish muscle in place of 0.15 ml blood serum. A 1-ml aliquot of chloroform extract was dried with nitrogen gas. To the dried residue was added 0.150 ml distilled water.

Muscle pH was estimated by measuring pH of a 5-g sample of white muscle homogenized with 45 ml of distilled water.

Physical Analyses. A shear-force method first described by Lee (1983) and adapted for measurement of fish texture by Chou (1989) was used to measure the texture of raw and cooked rockfish muscle.

The percentage of water lost from raw muscle (drip loss) was measured by weighing stored fillets, which were blotted with paper towels before recording their weight(s), and subtracting this weight from the initial weight of the fillet before storage, dividing the result by initial weight, and multiplying times 100. The percentage of water lost as a result of cooking was measured by (1) chopping fillets into cubes approximately 0.5 cm on a side, (2) microwave cooking the chopped fish (70 g) in a 150-ml centrifuge bottle for 90 sec at 60% power (750 W), (3) centrifuging the cooked fish at 100 rpm for 20 minutes in a GSA rotor, (4) measuring the volume of free liquid in the supernatant, and (5) multiplying this number by 100/70.

A reflectance colorimeter (Metron Instruments, Inc., St. John's, Newfoundland, Canada) with a 45° measuring device was used to obtain Hunter-Gardner tristimulus coordinates, (L, a, and b values) for the white and dark muscle of fillets. The L value of white muscle was used as an index of muscle opacity.

Results and Discussion

Organoleptic Evaluation of Grades A and B of Rockfish. The texture, odor, appearance, and flavor of cooked rockfish held on ice for up to 7 days were evaluated by the trained sensory panel using the interval scale method. Figure 1 summarizes the statistical analyses of the results. The mean odor scores of samples stored 1–4 days were significantly different ($P < .05$) from samples held 5–7 days. The mean texture scores were the same for samples held 1, 2, and 3 days and different ($P < .05$) from the scores for fish held for 4–7 days. Likewise, the appearance of raw fish samples held for 1, 2, 3, and 4 days was significantly different ($P < .05$) from that of fillets held for 5, 6, and 7 days. The color and appearance of cooked rockfish fillets did not change significantly over the 7 days of storage.

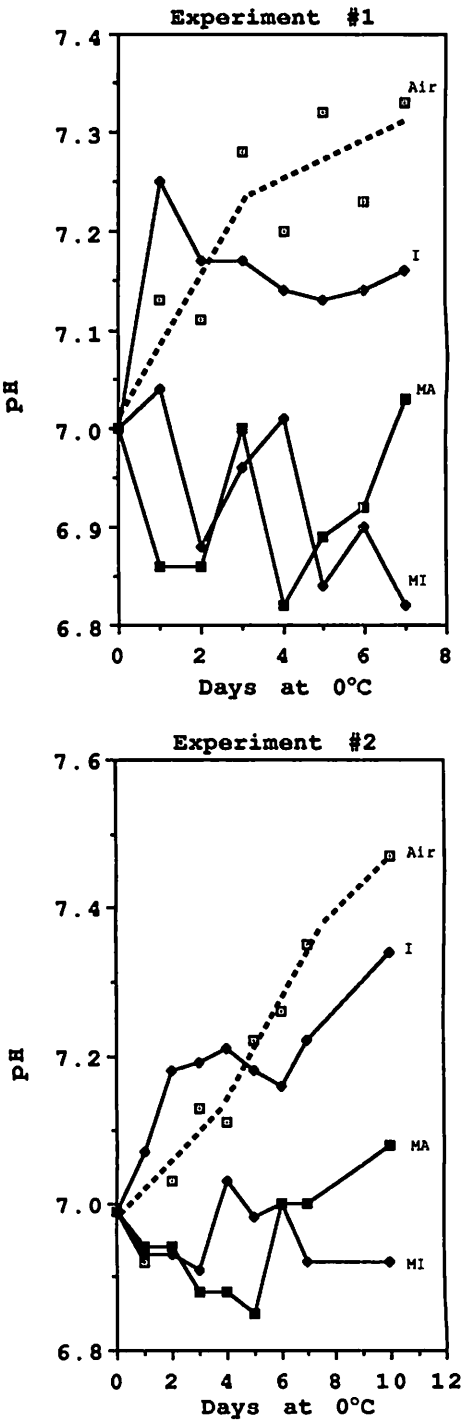


Figure 4. pH of fillets held at 0°C. Experiment #1 was with olive rockfish; experiment #2 was with yellowtail rockfish.

From these results, it was concluded that grade A quality of rockfish harvested by handline, immediately packed in ice, and filleted within 8 hours after harvest is defined by the odor and texture scores of cooked rockfish and the appearance of raw fillets stored for fewer than 4 days at 0°C. Grade B quality is associated with fish harvested and handled in the same manner and held on ice for 5–7 days. Table 1 summarizes the differences between grade A and grade B rockfish. The results of sensory analysis were similar for blue and yellowtail rockfish. However, the sensory characteristics used to distinguish grade A and B of blue and yellowtail were not applicable to chilipepper rockfish because the flavor and texture of the chilipepper fish were different (Yang, 1990).

Organoleptic Evaluation of Treated Rockfish Fillets. The sensory evaluations for raw appearance, cooked texture, cooked odor and overall acceptance of rockfish held in air, held in MA, treated with 1 kGy ionizing radiation and held in air (I), or treated with 1 kGy ionizing radiation and held in modified atmosphere (MI) at 0°C are summarized in Figure 2 and Table 2. The sensory attributes important to grade A quality deteriorated at a faster rate in treated samples than in the air control.

The deterioration in appearance of raw muscle subjected to the MA and MI treatments was associated with an increased opacity of the white muscle, which gave the tissue a cooked appearance. The appearance of fillets treated with 1 kGy radiation was unattractive because of a gray-brown discoloration of the red muscle. The MI treatment resulted in a market life for prime appearance of less than 1 day compared with 4 days for rockfish held in air. The increased opacity of fish muscle after harvest is caused by increased light scattering and appears to be the result of changes in the alignment and spacing of muscle fibers and myofribrils (Haard, 1992). On the other hand, dark discoloration of myoglobin-containing tissue in a CO_2-rich environment is caused by the acceleration of myoglobin oxidation to metmyoglobin (Wolfe, 1980).

The odor of cooked fish that had been treated with ionizing radiation deteriorated more rapidly than the odor of other samples. Panelists detected a characteristic off-odor in irradiated samples, particularly MI samples. This off-odor may be caused by radiolytic degradation products such as dimethylsulfide, which cause a "clamlike" off-odor (Miyauchi, 1960; Mackie, 1986). The results differ from those reported by Slavin et al. (1966), who found that doses of 1–2.5 kGy did not alter the normal characteristics of Atlantic rockfish. On the other hand, Licciardello et al. (1984, 1986) reported that Atlantic cod treated with 1 kGy of ionizing radiation had less acceptable odor than controls during the early stages of storage at 0°C.

The moist, succulent consistency characteristic of very fresh, prime-quality rockfish also was lost more rapidly in treated samples than in control samples held in air. Storage of muscle tissue in a CO_2-enriched environment normally lowers pH by a few tenths of a pH unit (Parkin et al., 1982), and it is expected that a lower flesh pH will cause a decrease in bound water as the pH approaches the isolectric point of myofibrillar proteins (about pH 5.5). Interestingly, the MI treatment (MI) resulted in an immediate deterioration in succulent texture as judged by panelists, and the overall sensory grade of these samples closely paralleled texture sensory scores (Figure 2).

Our finding that the MI treatments essentially abolished grade A market life of rockfish is consistent with the findings of Licciardello et al. (1984, 1986), who concluded from their study of Atlantic cod, "Although it is conceded that various low-dose irradiation treatments extended the shelf-life, it appears that grade B quality life of the fillets was principally extended and not the grade A prime quality."

Microbial Analyses. Storage trials with fillets of blue and yellowtail rockfish revealed relatively low total aerobic plate counts (about 3000 and 1000, respectively) at the start of storage trials (Figure 3). The initial

Table 4. Physical Characteristics of Fillets at Time of Grade A to Grade B Transition

	Treatment			
Characteristic	Air	MA	I	MA + I
pH				
Experiment 1	7.3	7.0	7.2	7.0
Experiment 2	7.2	6.9	7.2	6.9
Texture (kg/25 g)				
Raw	29	29	30	32
Cooked	40	70	47	62
Drip (% muscle weight)				
Raw	1.3	1.9	0.9	0.8
Cooked	26	28	21	22
L value				
Dark muscle	52	45	48	48
White muscle	54	53	53	51
a value				
Dark muscle	2.1	6.8	1.6	3.2
White muscle	–3.7	–2.8	–2.9	–1.4
b value				
Dark muscle	8.4	11.2	7.6	8.4
White muscle	1.0	6.6	3.1	7.3

Note. Values are for Grade A market life as indicated in Table 2. MA = mixture of 80% CO_2 and 20% air. I = treatment with 1 kGy of ionizing radiation and storage in air. Experiments 1 and 2 were with olive rockfish and yellowtail rockfish, respectively.

bacterial counts were lower than normally would be found in fish filleted by using standard commercial procedures. Low initial counts of bacteria were achieved by thoroughly washing off the exterior of the fish and cleansing the surface and knife used for filleting. The 1-kGy dose of ionizing radiation lowered the total aerobic bacterial flora by about one log cycle. Similar results were obtained by Licciardello et al., (1984, 1986). Storage of fillets in MA decreased the growth rate of aerobic bacteria compared with samples held in air. These results are consistent with those of earlier studies (Parkin et al., 1982). Fillets subjected to the MI treatment had extraordinarily low numbers of aerobic bacteria throughout 7 days of storage at 0°C.

In two experiments, with olive and yellowtail rockfish, total anaerobic plate counts were relatively low, about 100 colony-forming units per gram of fillets at the start of storage trials (Figure 3).

Treatments with MA and ionizing radiation were both effective in preventing growth of anaerobic bacteria, and the MI treatment actually caused a decrease in anaerobic bacteria when fillets were stored for 7 days at 0°C. The transition from grade A to grade B occurred at a higher total anaerobic count for air-stored samples than for the treated samples (Table 3).

Incipient spoilage of iced fish is normally associated with total aerobic plate counts exceeding 10^6 organisms per gram of tissue (Connell, 1975; Olley and Thrower, 1978). On this basis, yellowtail and blue rockfish held in air retained acceptable quality for more than 7 days of storage (Figure 3). Although the market life of iced round or gutted fish as an acceptable product is normally about 10–15 days at 0°C (Howgate, 1985), it is expected to be less for fillets because the flesh becomes contaminated with microorganisms during filleting.

For fillets held in air, the transition from grade A to grade B was associated with total aerobic bacterial counts of 10^3–10^4 per gram of tissue (Table 3). Total aerobic bacterial counts at the transition from grade A to grade B were about one order of magnitude lower for irradiated samples held in air and about two orders of magnitude lower for irradiated samples held in MA (Table 3). Although the numbers of bacteria were decreased by MA and I treatments, the transition from grade A to grade B occurred more rapidly. Accordingly, it appears that biochemical and physical reactions, other than those associated with microbial metabolism, are responsible for the loss of prime quality in treated fillets.

Physical Measurements. The pH of olive and yellowtail rockfish fillets increased during 7 days storage at 0°C in air (Figure 4). Increased flesh pH during storage in air was also observed in rockfish by previous investigators (Lauder et al., 1970; Parkin et al., 1982) and is normally attributed to various deamination reactions by bacterial enzymes and by endogenous tissue enzymes. The pH of fillets held in MA decreased by

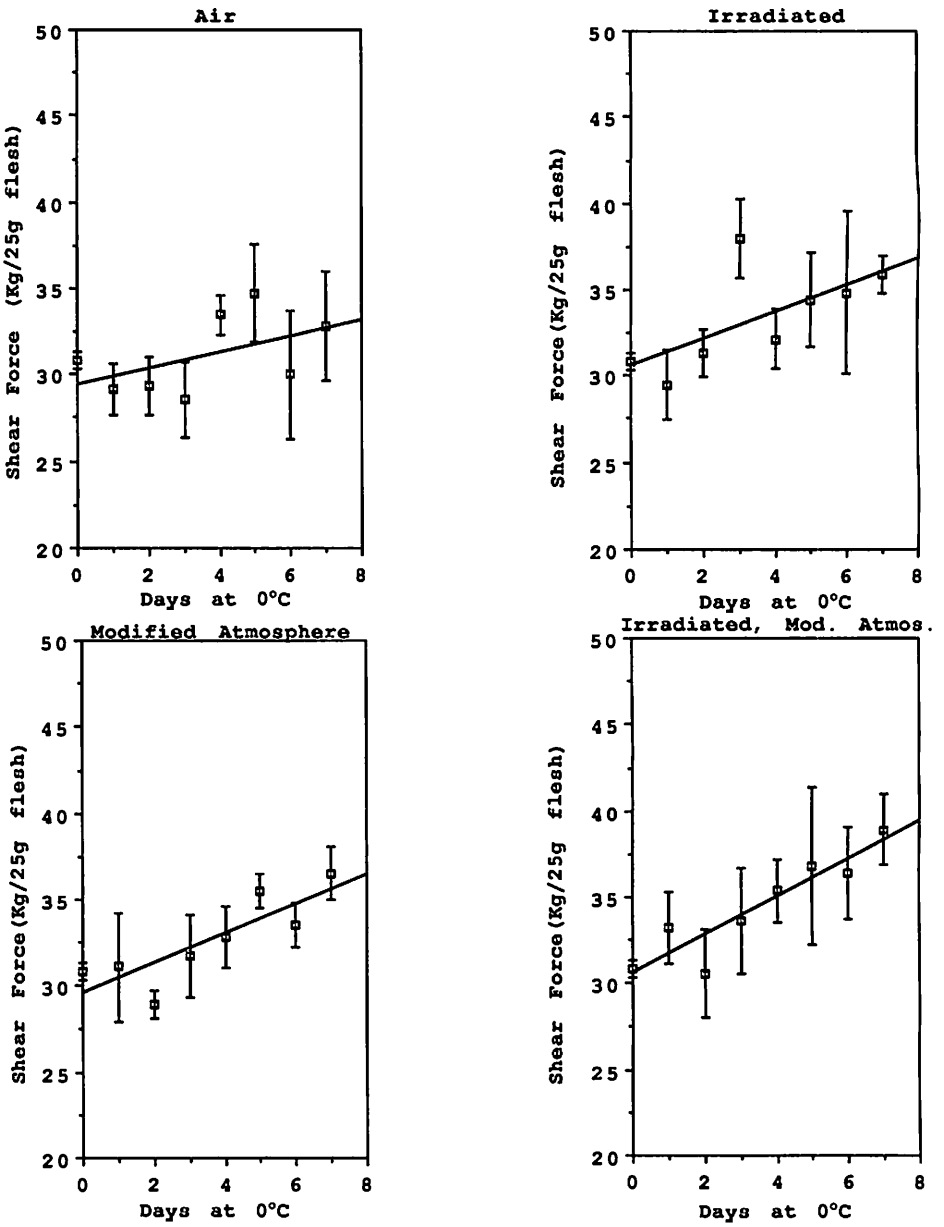


Figure 5. Texture of raw fillets held at 0°C. Each datum is average of duplicate determinations for each of three yellowtail fillets.

0.1 to 0.2 pH units during storage (Figure 4). The decline in pH of fish flesh during MA storage is due to the solubility of CO_2 as carbonic acid in the tissue (Parkin et al., 1982). The pH of irradiated flesh changed less than that of the unirradiated samples, possibly because of lower bacterial metabolism after irradiation (Figure 3). Similar results were reported for Atlantic cod exposed to 1 kGy of irradiation (Licciardello et al., 1984). The tissue pH of MA-stored samples was lower than that of air-stored samples at the time of grade A to grade B transition (Table 4).

The texture of raw, yellowtail rockfish flesh increased during storage of fillets at 0°C for 7 days (Figure 5). Earlier studies showed that the texture of yellowtail muscle became softer when round fish were stored on ice for 7 days (Chou, 1989; Kim, 1991). The texture of treated fillets increased in firmness, more so for MI fish than for other treatments (Figure 5). The change in raw texture was inversely related to the change in flesh pH (Figure 4); that is, after 7 days, MI samples had the lowest pH and firmest texture, whereas fillets stored in air for the same time had the highest pH and softest raw texture. The raw texture of fillets was similar for all treatment groups at the end of their grade A storage life (Table 4).

The texture of cooked rockfish became tough during MA storage (MA and MI treatments), unlike the samples held in air (Figure 6). The increase in firmness of the flesh was somewhat more pronounced in irradiated samples. Sensory scores for cooked texture showed a similar trend; that is, texture became dry and fibrous, and acceptability by panelists declined more rapidly for MA-stored samples than for air-stored samples. At the end of grade A market life, the texture of cooked fillets was significantly firmer for samples held in MA than for those held in air (Table 4).

Drip loss from raw yellowtail muscle increased during storage in MA and air. Exudation of liquid was greater for samples held in MA than for those stored in air (Figure 7). Statham and Bremner (1985) found that drip loss was positively correlated with the hydrogen ion concentration of the flesh. The water-holding capacity decreases as the pH approaches the isoelectric point of fish muscle (pH 5.6; Buttkus and Tomlinson, 1966). In contrast, Parkin et al. (1982) found little difference in weight loss of rockfish with respect to type of storage atmosphere. In a mixture of 25% CO_2 and 75% N_2, Greenland turbot lost less weight than in air at 0.1°C (Gauthier et al., 1986).

The liquid exudate from cooked flesh was greater than that from raw tissue (Figure 7). The water-holding capacity of MA-stored samples was lower than that of air-stored samples throughout the 7-day storage trial.

The water-holding capacity of irradiated samples was lower than that of unirradiated samples at the transition point of grade A to grade B (Table 4).

Reflectance colorimetry readings for dark and white muscle of olive

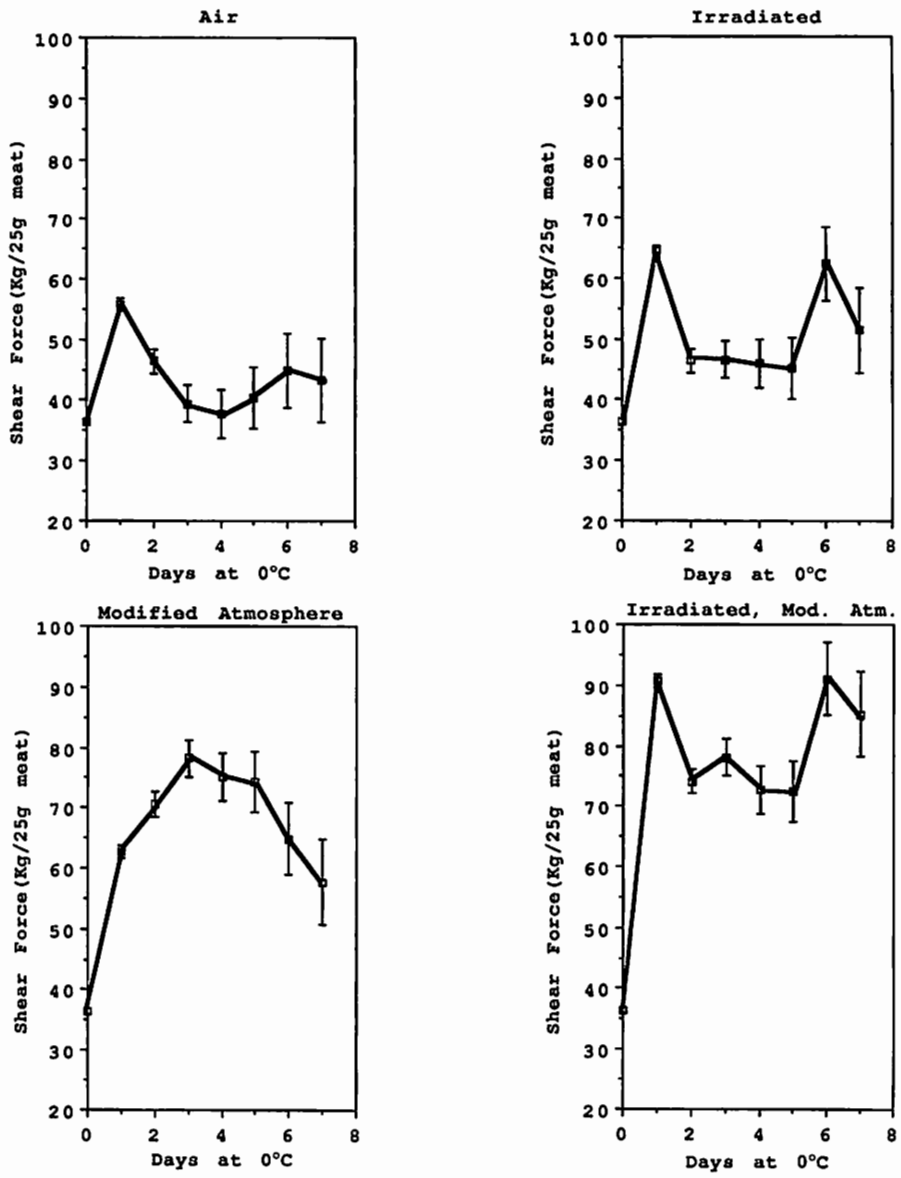
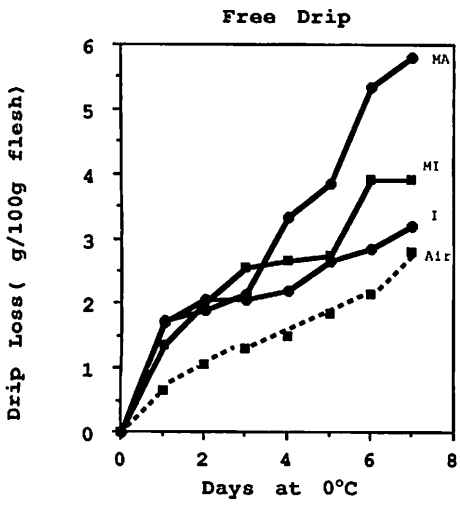


Figure 6. Texture of cooked fillets after storage at 0°C. Each datum is average of duplicate determinations for each of three yellowtail fillets.



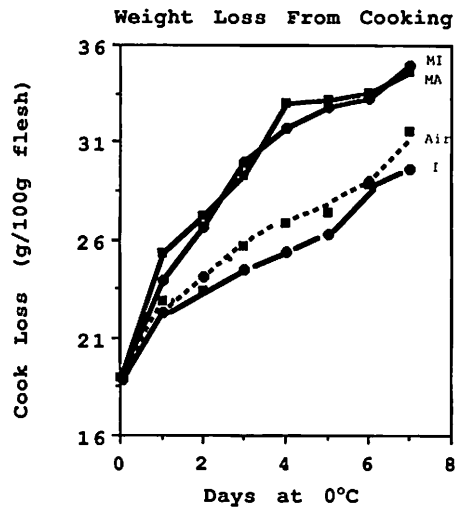


Figure 7. Loss of water-holding capacity in raw and cooked rockfish muscle after storage at 0°C. Data are average of duplicate determinations from two fish (yellowtail). I = 1 kGy ionizing radiation and storage in air; MA = storage in 80% CO_2 + 20% air; MI = 1 kGy radiation and storage in MA.

rockfish held under different storage conditions are summarized in Table 5. After 7 days storage, the L value of the white muscle increased, more so for MA samples than for fillets held in air (Figure 8). The increase in L value is associated with the increased opacity or cooked appearance of the raw flesh (Haard, 1992) and is consistent with the results obtained by sensory analysis in this study. The a value (redness) of white muscle did not change consistently during storage, but the b value (yellowness) tended to increase for all treatment groups.

The L value for dark muscle decreased during the first day of storage and did not appear to change consistently or differ among samples during the second to seventh days at 0°C. The a and b values for dark muscle both tended to increase during storage but were not consistently different between treatment groups. These results differ from those of sensory analysis; panelists noted a bright pink appearance of irradiated red muscle and darkening of MA-stored samples. The darkening of red muscle held in elevated CO_2 is caused by the formation of metmyoglobin. Gee and Brown (1978) reported that the decreases in L value and a value were associated with darkening of red muscle during storage in MA and could be prevented by addition of 1% CO to a modified atmosphere containing 80% CO_2.

Chemical Measurements. The TBA value gradually increased during storage for 7 days (Figure 9), more so for MA samples (MA and MI) than for the air control or irradiated samples. However, all TBA values were low, indicating that rancidity was not important throughout 7 days of storage on ice. Brown et al. (1980) also found low TBA values in Pacific rockfish that had been held in air or a mixture of 80% CO_2 and 20% air. Finne (1982) found that the tendency for swordfish to undergo

Table 5. Tristimulus Colorimetry of Fillets Held at 0°C for 7 Days

Dark Muscle

	L Value				a Value				b Value			
Day	Air	Air/I	MA	MA + I	Air	Air/I	MA	MA + I	Air	Air/I	MA	MA + I
0	52	—	—	—	0.2	—	—	—	2.7	—	—	—
1	46	48	45	48	4.3	1.6	3.4	3.2	5.0	7.6	7.2	8.4
2	47	44	45	47	4.6	5.7	6.8	4.8	9.8	5.7	11.2	6.1
3	51	45	47	47	2.1	3.8	5.0	4.2	8.4	10.8	10.4	11.4
4	48	39	45	47	4.0	7.7	4.3	5.5	8.0	7.1	8.5	11.5
5	51	46	53	52	1.3	3.6	2.3	0.8	7.8	10.8	9.4	7.9
6	45	50	45	49	6.1	4.9	7.2	4.9	6.0	1.8	9.0	12.5
7	45	45	51	43	6.2	3.7	3.6	5.7	7.7	14.8	13.5	11.9

White Muscle

	L Value				a Value				b Value			
Day	Air	Air/I	MA	MA + I	Air	Air/I	MA	MA + I	Air	Air/I	MA	MA + I
0	55	—	—	—	-3.4	—	—	—	1.8	—	—	—
1	52	53	49	51	-1.7	-2.9	-0.2	-1.4	6.8	3.1	5.5	7.3
2	57	52	53	55	-2.0	-2.0	-2.8	-3.0	5.3	5.0	6.6	3.9
3	54	53	52	53	-3.7	-2.5	-3.7	-2.4	1.0	3.6	5.4	6.8
4	55	51	53	57	-2.7	-2.2	-2.5	-2.9	6.0	4.9	5.4	6.2
5	55	56	57	56	-2.8	-2.9	-3.1	-3.5	6.7	3.4	5.7	6.0
6	54	55	57	59	-2.9	-2.0	-2.8	-3.1	4.7	8.1	9.1	6.9
7	55	57	60	58	-3.1	-3.1	-3.9	-3.6	3.9	4.7	5.9	6.7

Note. Data are average of five readings from each of two fish samples. I = treatment with 1 kGy of ionizing radiation. MA = storage in a mixture of 80% CO_2 and 20% air. Dashes = not done.

oxidative rancidity in a CO_2-enriched atmosphere is directly related to the O_2 concentration in the modified atmosphere. Likewise, 100% CO_2 or a mixture of 80% CO_2 and 20% N_2 was effective in preventing the rapid rise in TBA value that occurs in sardines held in air at 5°C (Fujii et al., 1989.

The free fatty acid content of white muscle increased slightly during the early stages of storage in air but did not significantly change during storage in MA (Figure 10). Similar results were obtained with dark muscle (data not shown). The increase in free fatty acids in dark muscle held in air was similar to that observed for white muscle (4 µmol per gram of tissue). The results indicate that MA is favorable to air storage with respect to hydrolytic rancidity. The thesis that the acceleration of texture toughening in MA is caused by free fatty acids is not supported by this experiment.

The major free fatty acids detected by gas chromatography in all samples were palmitic (16:0), ecosapentaenoic (20: 5), and docosahexaenoic (C22: 6) acids. The concentration of palmitic acid paralleled the results obtained for total titratable acidity. Because ecosapentaenoic acid and docosahexaenoic acid are highly unsaturated fatty acids that are susceptible to oxidation, the ratio of these fatty acids to palmitic acid during storage was calculated (Figure 10). The C22: 6/C16:0 ratio appears to be somewhat lower for MA samples compared with samples held in air. A lower ratio of C22:6/C16:0 is consistent with the occurrence of lipid oxidation.

The solubility of the myofibrillar proteins in 5% NaCl containing 0.02 M $NaHCO_3$ decreased only slightly during storage at 0°C for 7 days (Table 6). Protein extractability was significantly lower ($P < .05$) for irradiated (days 3–7), MA (days 1, 2, 3, 6, 7, 12, and 18), and MI (days 5–7) samples. The differences in protein extractability were relatively small during the first week of storage; however, extended storage for up to 18 days showed a pronounced decline in protein solubility in MA samples. Similar results were

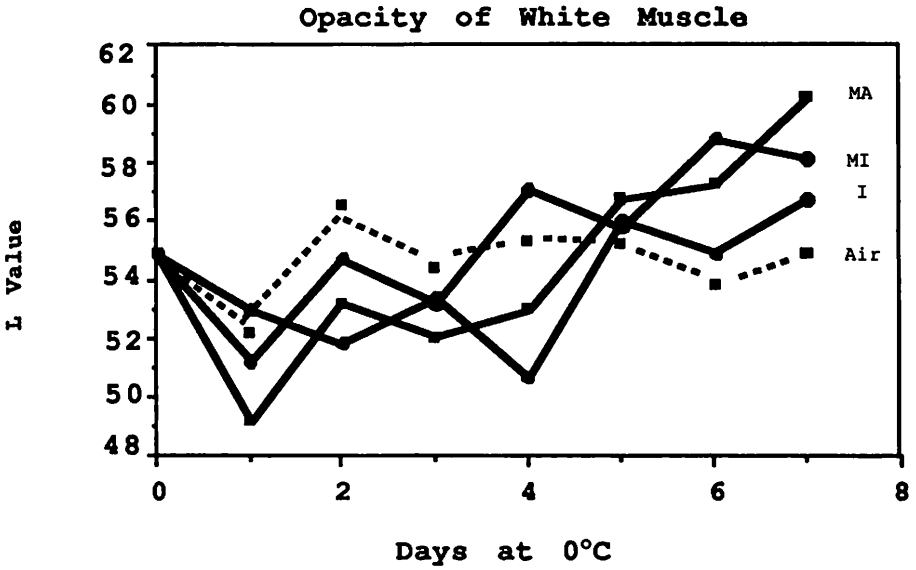


Figure 8. Tristimulus "L" value of raw white muscle from rockfish fillets held at 0°C. Data are average of five readings from each of two fish.

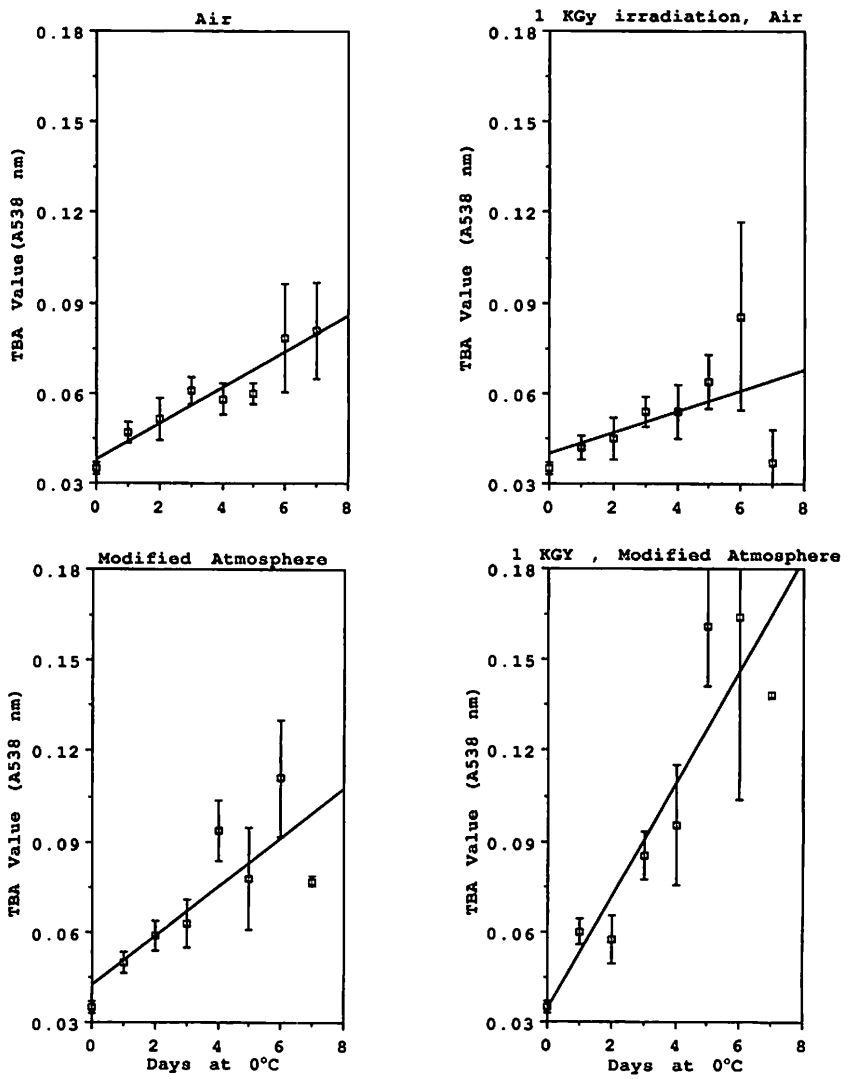


Figure 9. Lipid oxidation in rockfish fillets stored at 0°C, n = 4; bars are standard deviations.

reported by Collins et al. (1980), who compared black rockfish held in CO_2-modified refrigerated seawater and in ice. The loss in protein solubility in fish muscle is normally associated with development of dry, fibrous texture of the cooked product and is thought to be caused by formation of free fatty acids by lipases or by formation of DMA and formaldehyde by the action of TMAO demethylase on TMAO (Haard, 1991a).

Formation of DMA and formaldehyde from the TMAO demethylase reaction is associated with loss of protein solubility and texture deterioration in frozen fish. Analysis of rockfish held at 0°C showed that the concentration of DMA remained at low levels (0.29–0.69 mg N/100 g), characteristic of freshly killed fish for treatment groups. Similar results were found by Collins et al. (1980), who compared black rockfish held in ice or CO_2-modified refrigerated seawater for up to 14 days. On the other hand, Licciardello et al. (1984) noted a lower rate of DMA formation in Atlantic cod that were treated with 1 kGy and held in a mixture of 60% CO_2 and 40% air compared with fish held in air. It is well recognized that gadoid fish such as Atlantic cod have greater TMAO demethylase activity than nongadoid fish like rockfish.

Experiments to determine the concentration of ATP, ADP, AMP, IMP, inosine and hypoxanthine and the K value in rockfish held in air, in MA, and treated with ionizing radiation had not been completed at the time of this writing.

Conclusions

This study shows that treatment with 1 kGy ionizing radiation, and/or storage in MA (a mixture of 80% CO_2 and 20% air) decreases the number of aerobic and anaerobic spoilage bacteria in rockfish compared with untreated samples held in air. However, sensory evaluation of stored rockfish by persons trained to distinguish grade A and grade B quality showed that treatment with ionizing radiation and/or MA storage decreased grade A market life. Sensory evaluation further showed that both MA and I treatments caused

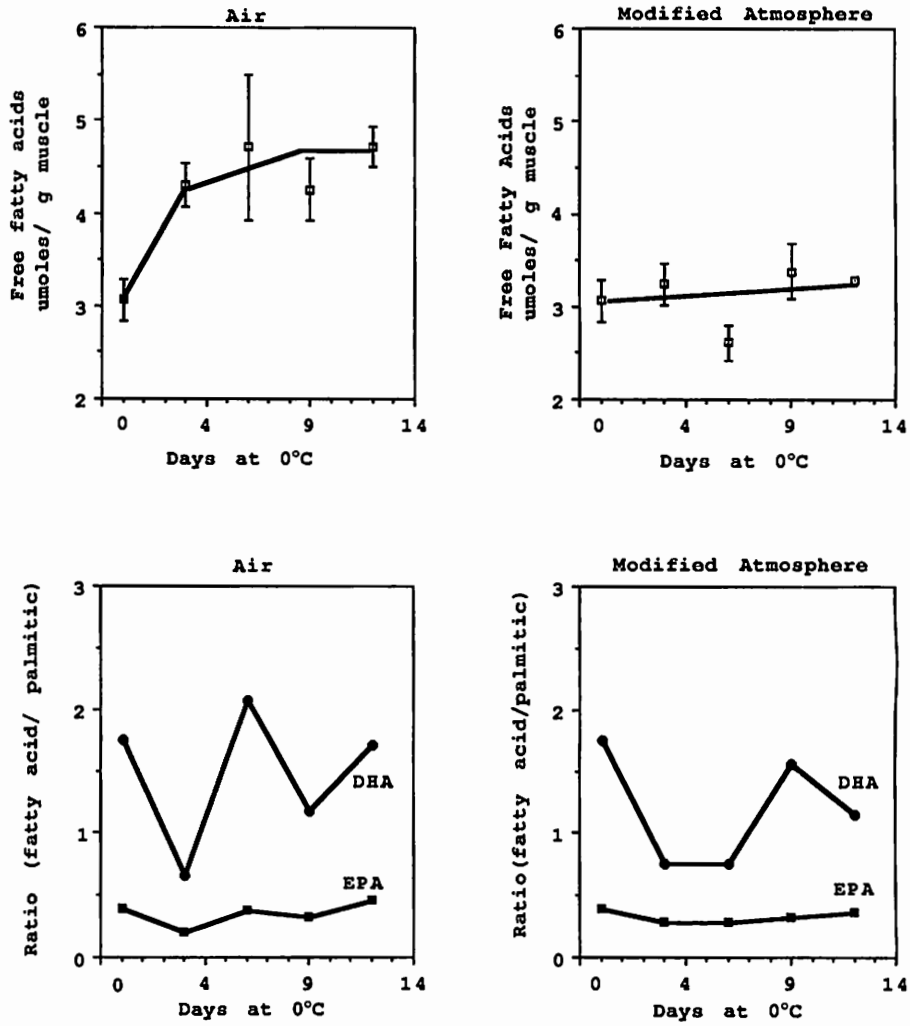


Figure 10. Free fatty acid formation in yellowtail rockfish held in air and modified atmosphere at 0°C. DHA = docosahexaenoic acid (C22:6); IPA = eicosapentaenoic acid (C20:5).

Table 6. Extractable Myofibrillar Protein from Rockfish White Muscle Held at 0°C

Days	Air	I + Air	MA	I + A
0	67.6	—	—	—
1	68.5a	66.9a	66.3	69.0a
2	68.1a	68.5a	66.7	67.0a
3	67.9a	65.2b	66.5c	67.4a
4	66.1a	64.5b	66.6a,c	67.8a
5	67.2a	64.1a,b	67.6a	63.6b
6	64.3a	68.3	64.0a,b	65.6b
7	69.8a	64.8b	64.1b	64.6b
12	65.3a	—	43.3	—
18	59.3a	—	29.8	—

Note. Data are average of five replicates for one experiment with yellowtail rockfish fillets. Values are percentage of extractable myofibrillar protein. Values in the same row followed by the same letter are not significantly different ($P < .05$). I = treatment with 1 kGy of ionizing radiiation. MA = storage in a mixture of 80% CO_2 and 20% air. Dashes = not done.

the texture of cooked product to become dry and fibrous. Moreover, samples pasteurized by ionizing radiation had an off-odor immediately after treatment. Objective measurements of rockfish quality showed that MA storage results in toughening of both raw and cooked fillets, loss of water-holding capacity of raw and cooked product, decreased flesh pH, decreased extractable myofibrillar protein, increased rate of lipid oxidation, and decreased rate of lipid hydrolysis. Additional research to understand why MA storage causes the texture of rockfish fillets to become dry and fibrous would help understand why grade A market life is adversely affected by this treatment.

Cooperating Organizations

National Marine Fisheries Service, Tiburon

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Temporal and Spatial Variation in the Species Composition of the Deep Water Eureka Trawl Fisheries, with Emphasis on Sablefish

Humboldt State University
R/NP-1-18H and R/F-131
Project Initiated: May 15, 1989
Project Completed: March 31, 1991

D. G. Hankin

In the jurisdiction of the Pacific Fishery Management Council (PFMC), use of groundfish bottom-trawling gear in deep water (>250 fathoms) has seen its greatest development in the Eureka, California, area. Principal species harvested in the "deep water complex" include dover sole, sablefish, and thornyheads (shortspine and longspine). Dover sole may be fully exploited in the Eureka area, and the weak market for deep water dover sole, which are often "jellied" (Fisher et al., 1987), limits landings from deep water. Sablefish is a highly valuable and moderately fast growing species that is thought to be fully exploited throughout the Council's jurisdiction. Allocation of limited sablefish quotas among longline, pot, and trawl gear has posed complex regulatory problems for the PFMC. Substantial commercial harvest of thornyheads has only taken place for the past decade or so, but a recent stock assessment indicated that thornyheads are slow growing and very long-lived (maximum age may exceed 100 years). The optimal (annual) instantaneous fishing mortality rate (F) for thornyheads may be as low as 0.02, in contrast to that for sablefish, which may be closer to about 0.15 (PFMC, 1990a).

Over the past several years, the PFMC has set complex regulations that have been designed to (1) extend the trawl fisheries throughout the year, (2) achieve the desired allocation of sablefish among user groups and restrict the landed sablefish catch to the allowable quota, and (3) (beginning in 1991) reduce the fishing mortality rate for thornyheads. Recent regulations, for example, have restricted landings of the deep water complex to a weekly limit of 27,500 lb, of which no more than 25% may be sablefish, and no more than 7500 lb may be thornyheads (PFMC, 1990b, Table 7). Fishermen are thus required by law to achieve a very specific mix of species in their landed catch if they wish to maximize landings. In many cases, this mix can only be achieved through substantial discard of restricted species, in particular, sablefish. Although PFMC data systems generate reliable records of total landed catches, the number of discards is generally unknown, and data on discards have rarely been gathered from commercially operating trawl vessels (Pikitch et al., 1988).

Regulation-induced discard of commercially valuable species is of substantial management concern, especially in the deep water trawl fisheries. However, little research has been devoted to fishing depths in excess of 250 fathoms, particularly in the Eureka, California, area where deep water trawl fishing is most prevalent. The objectives of this study were to characterize the species composition of fishermen's catches in the Eureka deep water trawl fisheries and to document seasonal changes in catches and discards that may reflect seasonal shifts in species distributions, PFMC regulations, or market-imposed delivery limits for particular species.

Initial funding for this research was provided by the Fishermen's Marketing Association (representing trawlers) and the National Marine Fisheries Service. Additional funding was provided by California Sea Grant via project R/F-131.

Table 1. Number of tows sampled by depth zone, month and year. Depth zone designations are: I = 000-110 FM; II = 110-210 FM; III = 210-310 FM; IV = 310-410 FM; V = 410-510 FM; VI = 510 + FM.

		Month											
Depth Zone	Year	O	N	D	J	F	M	A	M	J	J	A	S
I	89												
	90												2
	91				1								
II	89									1	1	2	2
	90										2		1
	91												
III	89	1	5										4
	90			2				3	1	1		3	2
	91				1								
IV	89		4								8	1	2
	90					1					3		
	91				1								
V	89		5	4							1	2	1
	90		3			1	4		4			3	5
	91				1								
VI	89	5	6							5	1	6	
	90		6				4	5		6		2	5
	91			4									
Totals		6	29	10	4	2	8	8	5	13	16	19	24

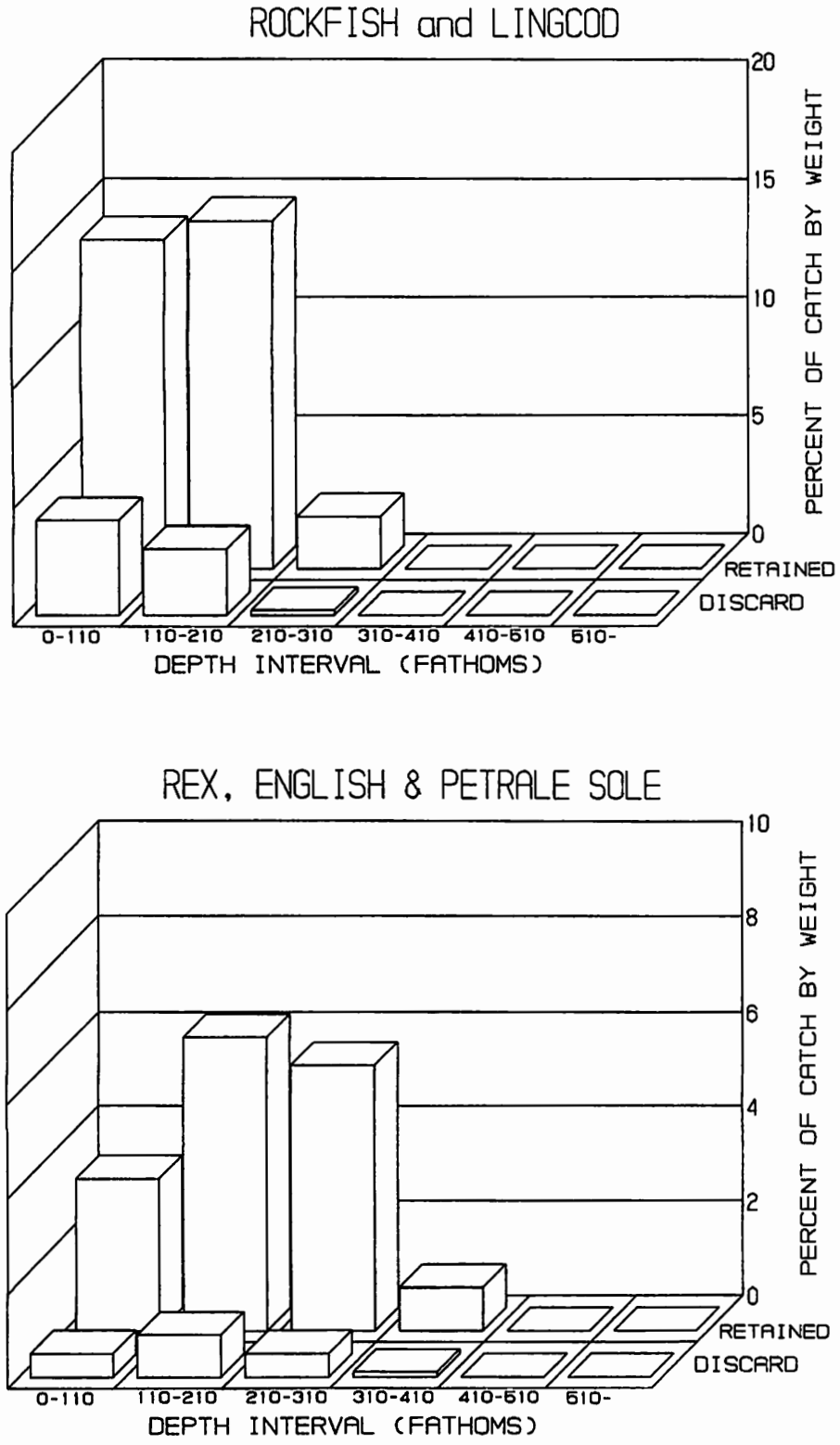

Figure 1. Mean percentages of rockfish and lingcod (top) and rex, English, and petrale soles (bottom) among retained and discarded catch by depth interval of catch. Plotted data are simple averages of catch percentages over all sampled tows.

Methods

Through a cooperative arrangement with the Fishermen's Marketing Association, a Sea Grant trainee (Eric Logan) carried out research on board Association vessels from Eureka and Crescent City, California. From June 1989 through January 1991, 29 productive trips were made on commercially operating vessels, for an average of about one and a half trips per month. The average length of a trip was about 3 days. A total of 207 tows were made during these trips; of these, 144 were carried out at depths of interest, produced fish, and were sampled for species composition, and so forth (Table 1). The average duration of a tow in deep water (>300 fathoms) exceeded 8 hours; for tows made at depths beyond 500 fathoms, average duration exceeded 10 hours.

Tow set and haul locations, time and depths of tow, and type and size of net were recorded for all sampled tows. Two samples of about 75 kg each were drawn from the total haul after codend contents were emptied into deck bins; a shovel was used to fill collecting baskets. These samples were used to determine percentage species composition and discard (by weight and numbers) for important commercial species and for incidental species and to determine between-sample variation in these estimates. Length frequencies of a large unsexed sample, or of all fish caught, were tabulated for sablefish on about 60% of sampled tows. Total tow weights were estimated visually for each sampled tow on the basis of apparent haul volume. As a check on the accuracy of these visual estimates, we obtained load cell measurements of total tow weights from 23 tows, thus allowing adjustment of visual estimates for possible bias. Weights of landed species and discards for most trips were estimated as the sum (over all tows) of the products of estimated sample percentages and (visually) estimated haul weights. These estimates were later compared with delivery records for landed species that were obtained for all species or species groups (e.g., thornyheads) at the end of trips at time of deliveries.

We also recorded any processor-imposed delivery limits (e.g., maximum number of pounds of deep water dover sole) that were in effect at the time of trips.

Results and Discussion

The results presented here are based on a preliminary analysis of data collected in our research project and are therefore general summaries. No analyses of seasonal trends are presented. More detailed analyses of collected project data will form the basis of a master's thesis for the Sea Grant trainee.

Catch and Discard By Depth. Although relatively few tows from shallow depths (<250 fathoms) were sampled, data from these tows, when compared with data from more extensive tows from deep water, provided useful descriptions of trends in species composition of retained and discarded catch for various depths. Catches of rockfish and lingcod were largely restricted to depths less than 200 fathoms, and catches of rex, English, and petrale sole were generally restricted to depths less than 300 fathoms (Figure 1). Catches of sablefish and dover sole were most substantial at intermediate depths (200–400 fathoms), but fish were present at all depths. Catches of shortspine and longspine thornyhead showed striking between-species differences in depth distribution. Although both species were most abundant in deep water (>300 fathoms), shortspine thornyhead were also captured in shallow waters, whereas longspine thornyhead were almost never caught at depths less than 300 fathoms (Figure 2). Although not substantial in most tows, Pacific grenadier accounted for more than 6% by weight of tows made at greatest depths (>500 fathoms); this species was essentially restricted to depths in excess of 400 fathoms.

In general, the species composition of deep water tows must be characterized as depauperate. At depths in excess of 300 fathoms, more than 80% of the total weight of catches could be accounted for by just five deep water complex species: dover sole, sablefish, longspine and

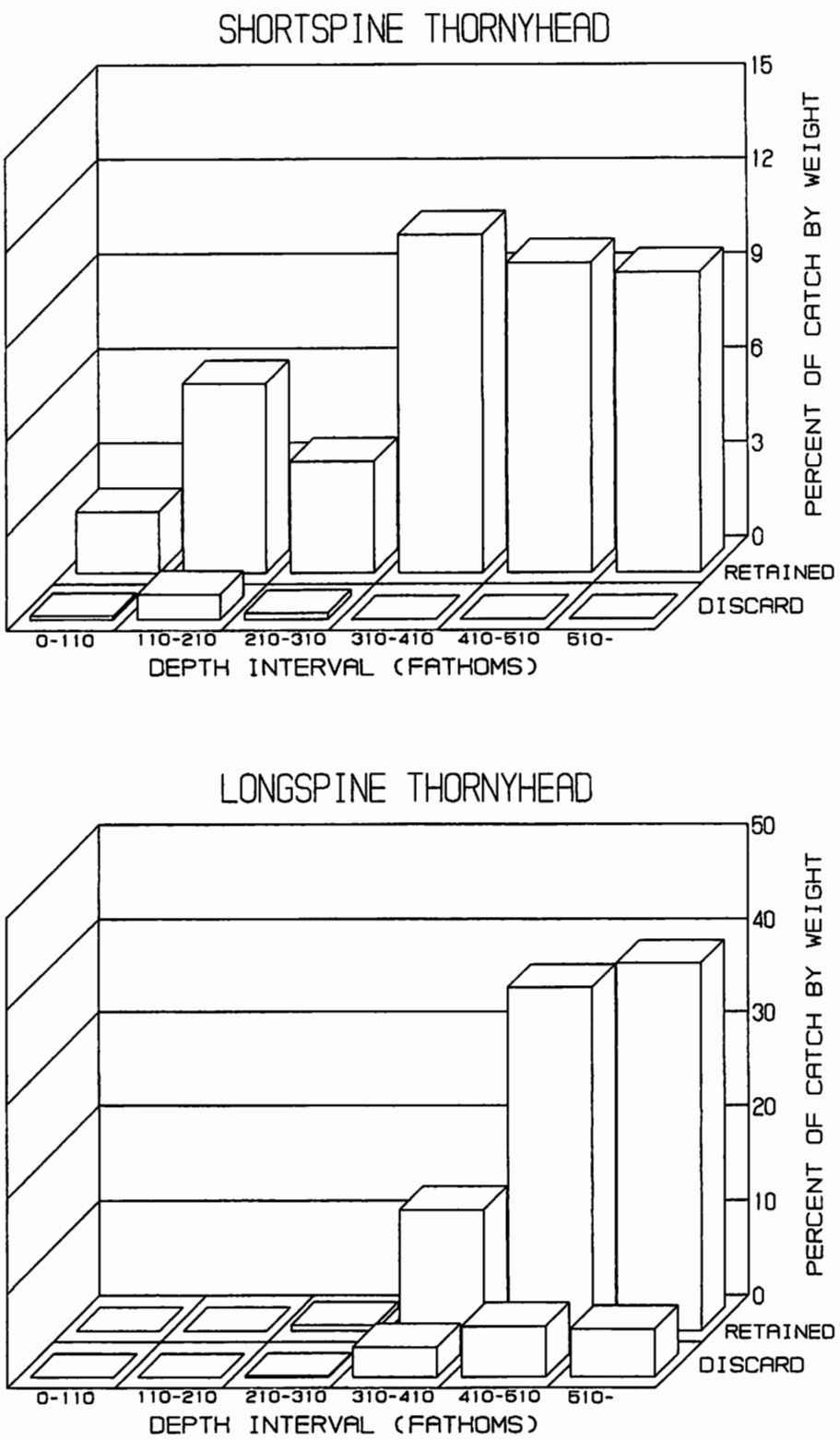

Figure 2. Mean percentages of shortspine (top) and longspine (bottom) thornyhead among retained and discarded catch by depth interval of catch. Plotted data are simple averages of catch percentages over all sampled tows.

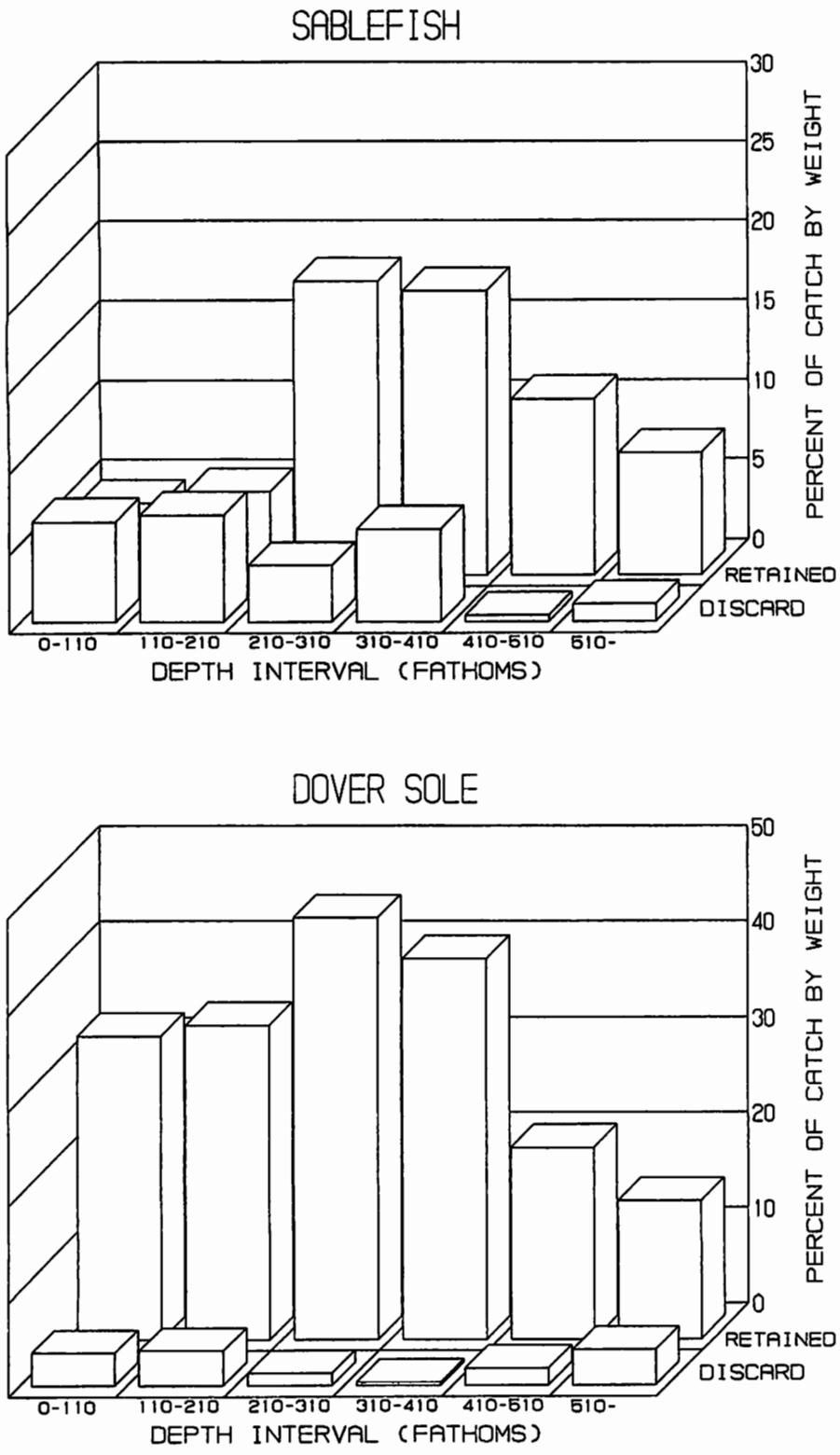

Figure 3. Mean percentages of sablefish (top) and dover sole (bottom) among retained and discarded catch by depth interval of catch. Plotted data are simple averages of catch percentages over all sampled tows.

shortspine thornyhead, and Pacific grenadier.

Discard patterns varied markedly among the four principal members of the deep water complex and appeared to be related to species-specific changes in depth distribution by size, fishery regulations, and market-imposed minimum size limits and delivery limits. Sablefish discard was greatest in shallow waters (Figure 3), where lengths of sablefish were generally small (average, about 45 cm) and often below the minimum size limit (Figure 4; see also Fujiwara, 1985). However, even though the average length of sablefish (about 55 cm) found in deep water (>400 fathoms) was substantially greater than that of sablefish from shallow water (Figure 4), the average weight of sablefish discarded at these depths was well in excess of any minimum marketable size (Figure 5). Although a few of the sablefish found in deep water were discarded because of their "poor appearance," it seems likely that most were discarded so that fishermen would not exceed their deep water complex landing restrictions of 25% by weight of sablefish. Sablefish of marketable size were also discarded by "high-grading," whereby fishermen retained only large-grade or large- and medium-grade sablefish, which are worth more per pound than small marketable sablefish.

The number of dover sole discarded was generally greatest in very shallow water (<200 fathoms) and in very deep water (>400 fathoms). Few were discarded at 200–400 fathoms, where dover sole were most abundant (Figure 3). Figure 6 shows that in shallow waters the average weight of individual dover sole discarded was substantially less than that of retained catch. At depths greater than 400 fathoms, the average weights of retained and discarded dover sole were essentially the same. In shallow waters, the dover sole discarded were principally fish below commercially valuable size. In deep water, discards (of larger fish) were the result of market-imposed restrictions on landings of deep water dover sole that have poor market

quality.

Average weights of discarded longspine thornyhead were much smaller than those of retained longspine thornyhead at all depths and reflect minimum marketable size limits imposed by processors. In contrast, discards of the larger shortspine thornyhead were negligible in deep water (>300 fathoms), where only larger fish were found; and in shallow depths (where longspines were absent), the average weights of smaller fish discarded were always much smaller than those of fish retained at those depths (Figure 7).

Overall, discard rates for deep water complex species appear to reflect a complex interaction of regulations imposed by the PFMC and by processors. Minimum size limits imposed by regulation (sablefish) or marketability (processors) seem principally responsible for discards of dover sole, sablefish, and shortspine thornyhead found in shallow water (<200 fathoms). In deep waters (>300 fathoms), discards of marketable-sized sablefish appear to be principally due to PFMC regulations that restrict the percentage composition of sablefish in deep water complex landings. Large-grade sablefish (>7 lb) command a higher price than smaller sablefish and were almost never discarded, whereas small-grade sablefish (<5 lb) were often discarded when the 25% sablefish limit was being approached. This high-grading of sablefish catch would tend to maximize vessel profits, given a limit on total sablefish landings. Discards of large dover sole caught in deep water are almost entirely due to processor-imposed delivery limits for this product.

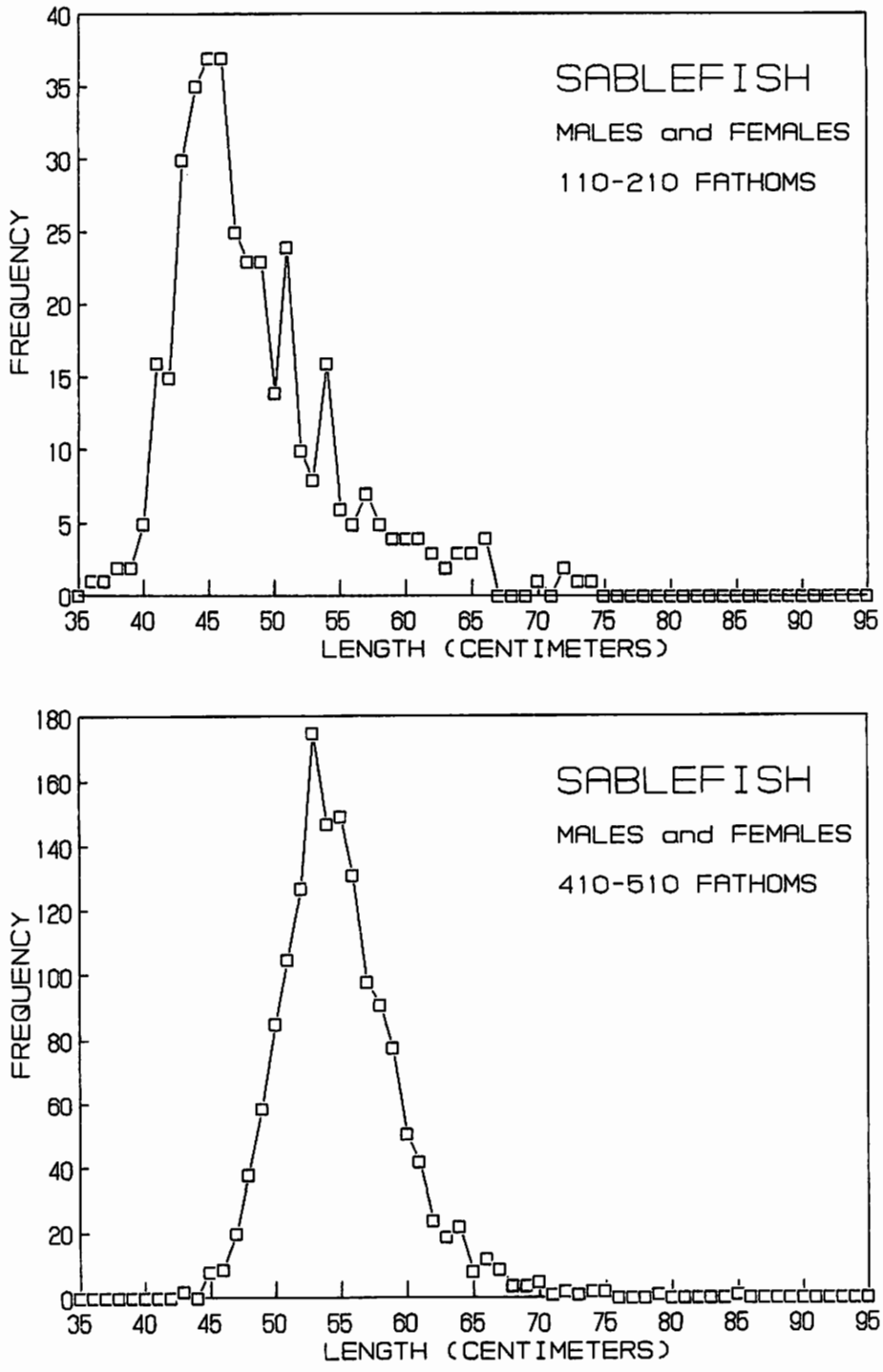

Figure 4. Length frequencies of unsexed sablefish from all tows sampled from 110–210 fathoms (top) and from 410–510 fathoms (bottom).

Load Cell Measurements of Haul Weight. Although visual (volumetric) estimates of haul weight were highly correlated with load cell measurements of haul weight (r = .84), we found that visual estimates of haul weight had an expected positive proportional bias of about 15% when compared with the accurate weight estimates. The Sea Grant trainee on this project had extensive previous experience in visual estimation of haul weights while serving as an observer for the National Marine Fisheries Service on several foreign fishing vessels, but had had little previous opportunity to validate estimates. Also, the differences between load cell and visual estimates of haul weights appeared to increase with increasing haul weight (Figure 8). Although Figure 8 will allow us to adjust for bias in visual estimates in final analysis of our own project data, these findings suggest that greater attention should be placed on accuracy of estimation of haul

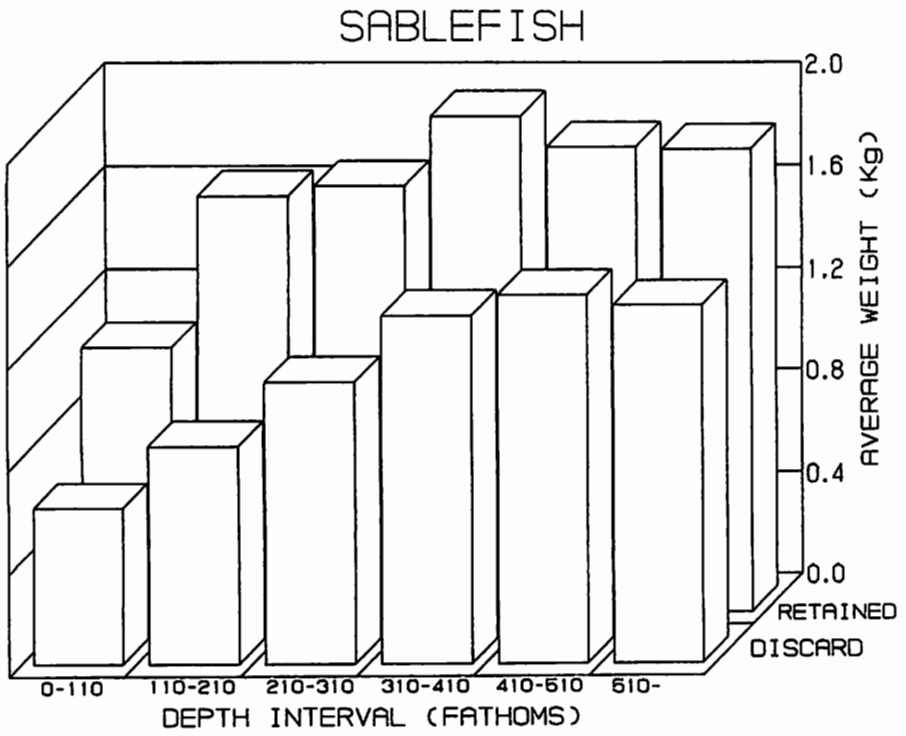

Figure 5. Average weights of individual unsexed sablefish retained or discarded plotted against depth interval. Based on simple averages of mean fish weights over all sampled tows.

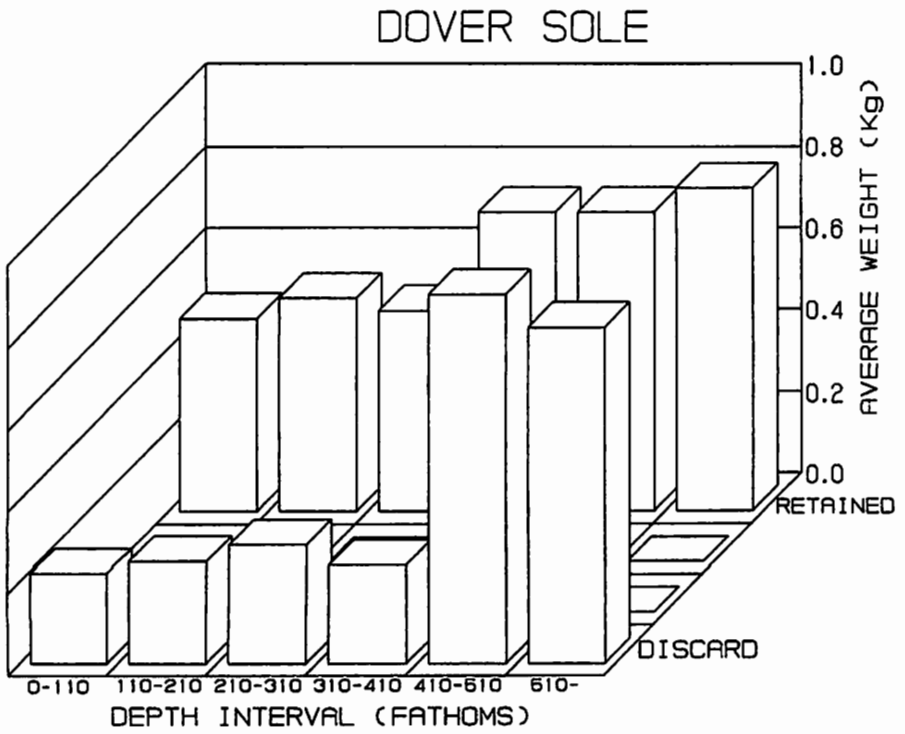

Figure 6. Average weights of individual dover sole retained or discarded plotted against depth interval. Based on simple averages of mean fish weights over all sampled tows.

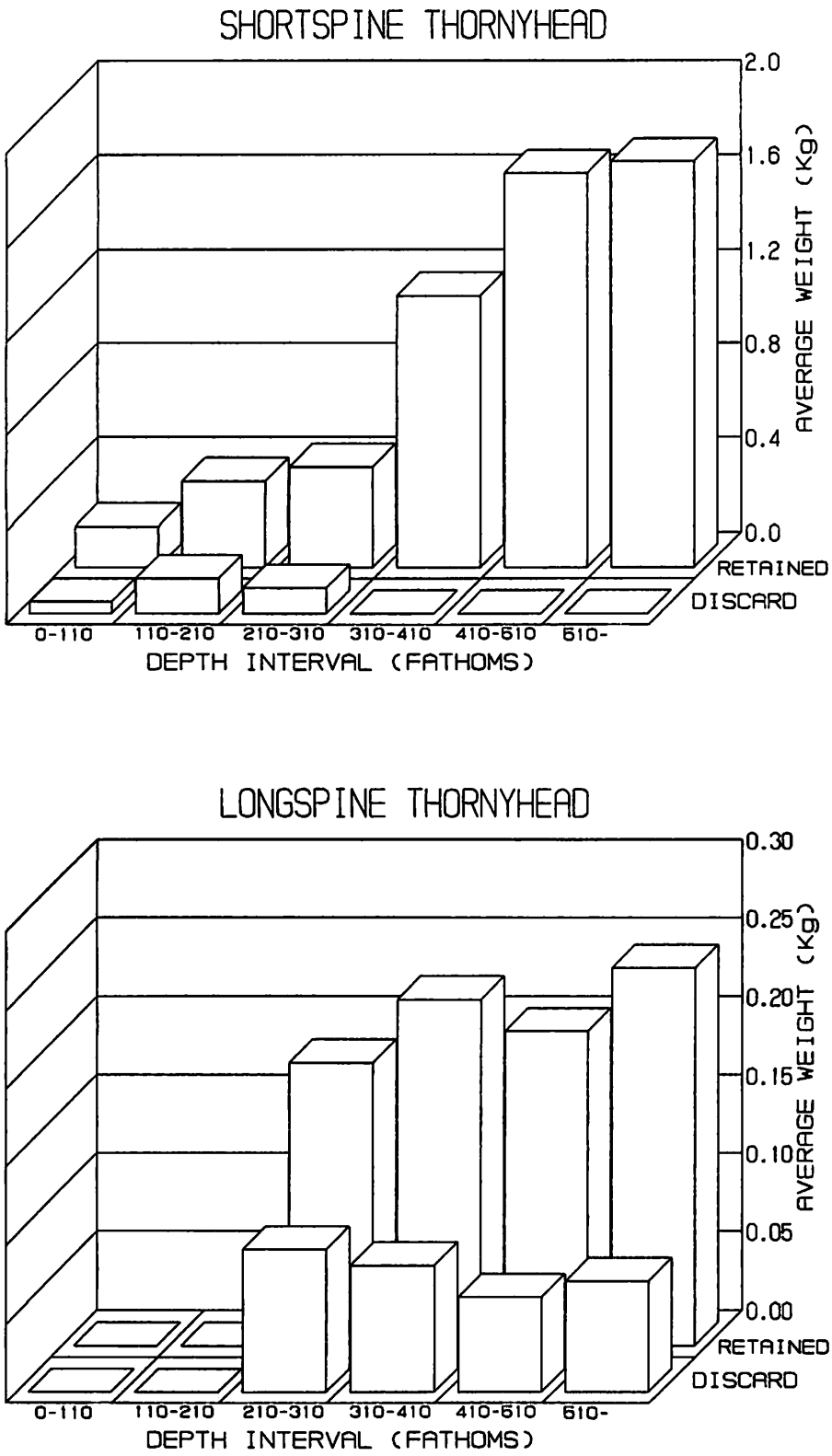

Figure 7. Average weights of individual shortspine (top) and longspine (bottom) thornyhead retained or discarded plotted against depth interval. Based on simple averages of mean fish weights over all sampled tows.

weights when visual methods are used (as in most trawl fishing vessel observer programs). To our knowledge, the 23 haul weights obtained with load cell in our project are the largest such collection of weighings made on board operating commercial trawl vessels. These weighings should in theory be accurate, but sea conditions could have affected load cell weights because of difficulties wtih the stability of the read-out device in rough seas.

Cooperating Organizations

Fishermen's Marketing Association, Eureka

Northwest and Alaska Fisheries Centers, National Marine Fisheries Service, Seattle, Washington

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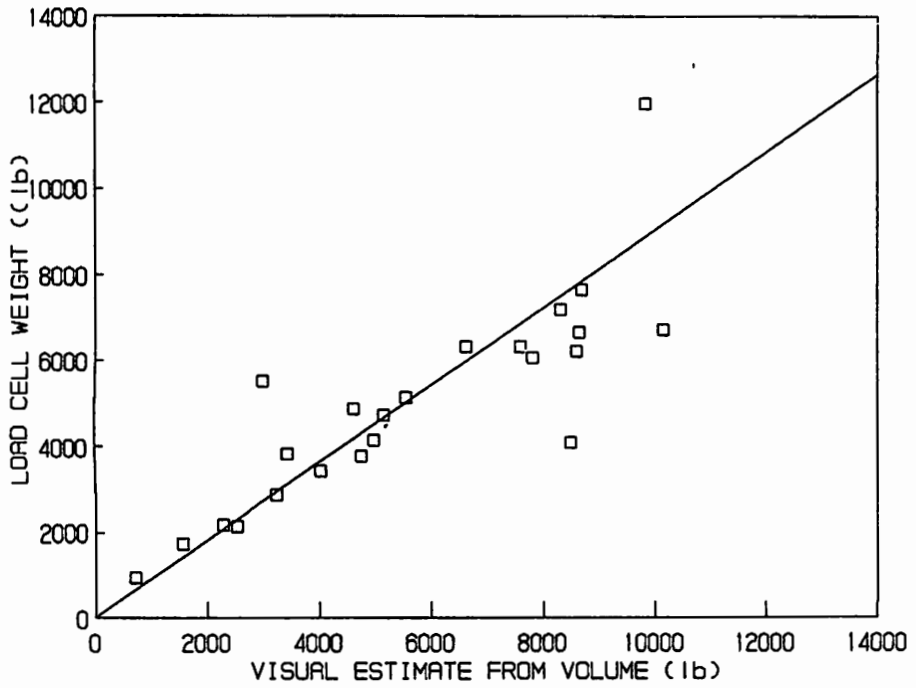

Figure 8. Load cell measurements of codend weights plotted against visual estimates of codend weight based on volume. Plotted line is forced through the origin (nonsignificant regression test for intercept) and the least squares estimate of slope is 0.850.

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New Marine Products

GABA-Mimetic Peptides from Marine Algae and Bacteria: A New Class of Potential Diagnostic and Therapeutic Agents

University of California, Santa Barbara
R/MP-37
Project Initiated: October 1, 1985
Project Completed: September 30, 1989

Daniel E. Morse and Aileen N. C. Morse

Our overall objective was to identify and develop potentially useful new peptides that act like gamma-aminobutyric acid (GABA) from marine algae and bacteria for improved diagnosis and treatment of brain diseases and other disorders of the central nervous system (Morse, 1985, 1986, 1988; Morse and Morse, 1988). We propose that a systematic analysis of the structure-function relationships of natural and synthetic variants of these GABA-mimetic peptides can be used to (1) probe and map the extended topological and binding determinants of GABA receptors; (2) identify those features that control the specificity and efficiency of ligand binding to GABA receptors; and (3) produce new compounds useful for therapeutic and diagnostic purposes.

To obtain sufficient material for these studies, we have characterized the levels of GABA-mimetic peptide produced in 25 different species and strains of marine red algae and cyanobacteria grown under a variety of physiological conditions. We have found that GABA-mimetic peptide synthesis is apparently "constitutive," (i.e., the level of specific peptide synthesis is not changed by alterations in light exposure [intensity, wavelength, or periodicity]; nitrogen source; other nutrients; or the phase of growth of the algae or bacteria). However, we have succeeded in identifying a mutant of the red alga *Gracilaria* (strain B-1, from van der Meer) and a strain of marine cyanobacterium (*Synechococcus* strain DC-2) that produce significantly higher (up to thirty-fold higher) quantities of the GABA-mimetic peptides than those produced by the wild-type and other strains with which we first began our investigations. Both of these high-level–producing strains can be grown axenically to high cell yields, thus optimizing production of the peptide-starting material.

Most recently, we have begun to use genetic engineering to further enhance the production of the GABA-mimetic peptides. We are now cloning specific genes from the red algae, in recombinant DNA plasmid and phage vectors, in order to amplify production of the desired products in bacterial cells. These efforts have been facilitated by the recent development (by Marina Roell, gradute student trainee) of improvements in the efficiency and yield of purification of RNA and DNA species fromn marine red algae (Roell and Morse, 1989).

Using these mutant algal strains that produce high levels of these peptides, we have developed an improved method for the extraction and initial purification of the GABA-mimetic peptides. This method efficiently and rapidly separates the peptides from toxic compounds that are present in the cyanobacteria and red algae. This initial step, employing heat-denaturaion of the toxins and proteins, followed by their removal by centrifugation and ultrafiltration, thereby facilitates both the quantitative and reliable assay of the GABA-mimetic peptides, and their further production. The desired peptides have then been further purified an additional 10,000-fold, using procedures of gel-filtration and high-pressure liquid chromatography (HPLC) we developed last year. The resulting oligopeptides have molecular weights ranging from 600 to 1,300 d.

Thus far, we have found that each of the GABA-mimetic oligopeptides contains a novel basic amino acid (the structure of which we are currently investigating) and a small number of residues of taurine, alanine, and glycine (in one of the most active algal peptides) or a comparable number of residues of glutamic acid and alanine (in one of the most active bacterial peptides). We found that these peptides are refractory to conventional techniques for Edman microsequence analysis, presumably because of their content of unusual residues and possible cyclic structures. Cooperative assistance from the Department of Chemistry at the University of Illinois (Urbana) and from Porton Industries (Tarzana, California) is now facilitating the structural analysis of these bioactive materials.

We have found that the most highly purified peptides bind strongly to GABA receptors isolated from mammalian brain and that significant differences in the strength of this binding are determined by differences in the structures of the algal and bacterial peptides. Measurements of radioactive ligand-binding competition to isolated GABA post-synaptic receptors purified from mouse brain reveal that the taurine-containing algal peptide binds to the brain receptors with an affinity that is about 100-fold greater than that of GABA itself; in contrast, the peptide that lacks taurine, which is purified from cyanobacteria, binds to these receptors with an affinity only equal to that of GABA. We presently are working to identify the principal structural determinants in the algal and bacterial peptide sequences that govern the strength of binding of these peptides to GABA receptors from mammalian brain.

Cooperating Organizations

Agouron Biotechnology Corporation, La Jolla, California
AMGen Corporation, Thousand Oaks, California
Beckman Corporation, City of Hope Medical Research Institute, Duarte, California
Porton Industries, Tarzana, California
University of California, Biotechnology

Research and Training Program (Systemwide)
University of Illinois, Department of Chemistry, Urbana, Illinois

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Marine Chemistry and Pharmacology
Program: Pharmacology

University of California, Santa Barbara
R/MP-38
Project Initiated: October 1, 1986
Project Completed: September 30, 1990

Robert S. Jacobs

Pseudopterosin E

We have now completed our studies of pseudopterosin E and are preparing the publications on this subject. United States and international patents have been obtained, and, in conjunction with Dr. William Fenical, we are investigating potential interest from members of the pharmaceutical industry in the United States and abroad. Pseudopterosin E is an unusual drug, capable of blocking degranulation and release of eicosanoids from polymorphonuclear neutrophils. Our experimental data also suggest that pseudopterosin E is a prodrug that targets cells containing a fuscosidase enzyme that activates the compound. The activated drug then inactivates phospholipase A_2 and the 5-lipoxygenase enzymes necessary for degranulation and production of leukotrienes. The net result is that acute inflammatory conditions, such as allergic reactions and skin irritation, can be blocked by administration of pseudopterosin E. We also have shown that this compound is systemically and topically active. It is my view that pseudopterosin E is sufficiently novel to warrant its development for use in conditions such as lung inflammation and psoriasis, as possible adjunct therapy in radiation treatment, and in burns, shock, and other conditions involving tissue infiltration of neutrophils and their degranulation in which this cellular reaction is detrimental to the person's survival. We are confident that with the publication of our results, interest in this drug with industrial scientists will lead to licensing options. Ed Luedke, our Sea Grant trainee, has submitted a thesis on this subject and is joining Merck Sharp and Dohme to pursue his career as a postdoctoral fellow in this area of research.

Fuscoside

Fuscoside is a new marine natural product that was isolated from the soft coral *Euniciae fusca* by Fenical's group. It contains an unusual glycoside that is active against inflammation induced by phorbol myristate acetate. In biochemical studies, this compound did not inactivate phospholipase A_2. However, in peritoneal macrophages and human polymorphonuclear neutrophils, fuscoside selectively inhibited formation of the eicosanoids leukotriene B_4 and leukotriene C_4, which are products of the 5-lipoxygenase pathway. The compound was ineffective in inhibiting formation of prostaglandins. In fractions of cell homogenates, fuscoside effectively inhibited 5-lipoxygenase conversion of arachidonic acid to leukotriene B_4. In studies with whole animals and a topical model for local anesthesia, the effects of fuscoside were persistent, lasting 24 hours after application. The long-lasting effectiveness was also observed in inflammation induced by phorbol myristate acetate. We think this drug may have an unusual mechanism of action, and we are evaluating it to determine if work should be continued toward its development.

Scalaradial

As a consequence of introducing our phospholipase A_2 assay in the field and into our focused screening program, we found a number of different types of phospholipase A_2 inhibitors in a variety of marine organisms. Scalaradial is a potent compound from a marine sponge. It differs structurally from manoalide. Scalaradial irreversibly inhibited phospholipase A_2 and had good topical and systemic antiinflammatory activity in mice. To rationally evaluate the mechanism of action of this drug, Marianne de Carvalho, a Sea Grant trainee, developed an approach in which gas chromatography and mass spectrometry are used to measure directly the release of arachidonic acid from macrophages. Consequently, the result of phospholipase A_2 inhibition in this important immune cell can also be measured directly.

Using these methods, she found that scalaradial directly inactivated cellular phospholipase A_2, resulting in the decreased release of all the eicosanoids without producing cellular toxicity. This is a significant improvement over manoalide, and if preclinical evaluation holds up, it is our hope that scalaradial or one of its analogues could be tested for clinical efficacy in arthritis. However, as with manoalide, development of this drug would take several years and an extensive commitment from a drug company. Wyeth-Ayerst is assessing whether scalaradial can serve as a lead compound in their program. Marianne has detailed her studies in her trainee report, and scalaradial is the subject of her doctoral thesis.

Manoalide

Work on manoalide has been restricted to special experiments designed to specify the ability of this drug to inactivate various forms of phospholipase A_2. Thus, in conjunction with Wyeth-Ayerst, we investigated the effects of manoalide on inactivation of phospholipase A_2 that had been isolated from synovial fluid obtained from patients with arthritis. This work required a detailed study of the kinetics of this enzyme and of its reactions with manoalide. Basically, as anticipated, manoalide was also effective in inactivating this pathological form of phospholipase A_2. Although manoalide itself may be too toxic for systemic use, this study reaffirmed our view that an inactivated form of this enzyme could have important clinical implications.

Screening New Compounds

During the 1989–90 Sea Grant fiscal year, we received 21 new marine natural products from three of our collaborating chemists (11 from J. Faulkner, 7 from W. Fenical, and 3 from P. Crews).

Of the 11 new compounds from J. Faulkner, one showed activity as an inhibitor of cell division in the sea urchin assay, 7 showed mild to moderate activity as antiinflammatories in the mouse ear edema assay, and 8 showed mild to moderate activity as inhibitors of bee venom phospholipase A_2.

Of the 7 new compounds received from W. Fenical, none showed activity as inhibitors of cell division in the sea urchin assay; 5 showed activity as antiinflammatories in the mouse ear edema assay, 2 of which were quite active; and 4 showed mild to moderate activity as inhibitors of bee venom phospholipase A_2.

The 3 new compounds received from P. Crews are novel derivatives of scalaradial and showed moderate activity as antiinflammatories in the mouse ear edema assay. Only 1 showed mild activity as an inhibitor of bee venom phospholipase A_2.

Some compounds received previously (1988–89) were also screened during the current fiscal year of this report. Three compounds from J. Faulkner showed high activity, and 6 showed moderate activity as antiinflammatories in the mouse ear edema assay. Five compounds from W. Fenical and 12 compounds from V. Paul showed moderate activity as antiinflammatories in the mouse ear edema assay. In addition, 11 compounds from V. Paul showed activity as inhibitors of bee venom phospholipase A_2; 3 showed almost complete inactivation of the enzyme.

New Developments

We have added a new anticancer model to our empirical screening program. This is a cell culture system composed of human and lower vertebrate cancer cells that are resistant to chemotherapy. Resistant cancer cells have a cell transporter protein that prevents cellular accumulation of the cytotoxin and, in patients with terminal cancer, may account in part for the fact that these patients no longer respond to treatment. The cells were provided to us by the National Institutes of Health (Michael Gottsman, Director, U.S. Public Health Service), and we have been trying to determine if any of our marine natural products can poison the transporter protein and thus make these cells sensitive to cytotoxins. Allen Williams, one of our graduate students, has begun this study. We have found a series of cyclic peptides that inactivate the P-170 pump mechanism in these cells. We are pursuing this lead. These compounds were submitted to us by Francis Schmitz and Chris Ireland. Bill Fenical and John Faulkner also have provided us with active leads. Thus, this new model is showing promise of providing new pharmacological compounds.

Cooperating Organizations

Allergan
SmithKline Beecham Pharmaceuticals
Merck Sharp and Dohme
Smithsonian Marine Laboratory
Harbor Branch Oceanographic Institution
Bristol Meyers

Publications

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Jacobs, R. S. TV conference through satellite to Japan, Higashi-Nippon Broadcasting, Co., Ltd., January 23, 1991.

Jacobs, R. S. Seminar, United States Department of Commerce, NOAA, Silver Springs, Maryland, May 17, 1991.

Jacobs, R. S. Spring Biochemistry Conference on Prostaglandins, Leukotrienes, Lipoxins and PAF, George Washington University, Washington, D.C., May 13–17, 1991.

Jacobs, R. S. Seminar, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania, May 20, 1991.

Marine Chemistry and Pharmacology Program: Development of New Drug Leads from Marine Plants and Gorgonian Corals

University of California, San Diego
R/MP-39
Project Initiated: October 1, 1986
Project Completed: September 30, 1989

William Fenical

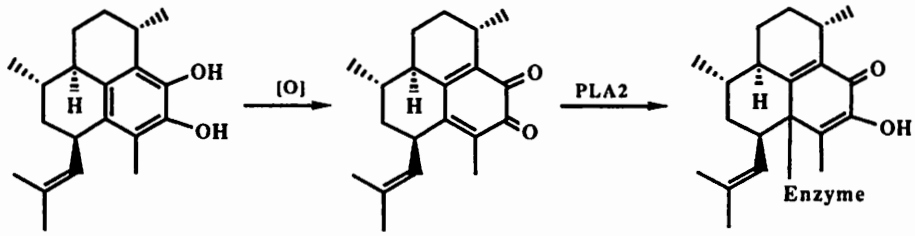
During the past 36 months, the goals of this project were to explore marine plants and animals for new leads in the treatment of inflammatory disease and related problems, as well as to further explore existing discoveries with a major emphasis on the elucidation of novel mechanisms of pharmacological action. Major expeditions to the Philippines and western Australia to acquire samples for study were involved, and several new leads were discovered. Two ship-board expeditions to the Bahama Islands were particularly fruitful in locating three new classes of antiinflammatory-analgesic compounds. Working with Jacobs' research group, we have provided convincing evidence of a novel mechanism of action for pseudopterosin E (PsE). This information will greatly facilitate the development of PsE as an antiinflammatory drug. Details of these studies, particularly the last 12 months of research, follow.

New Drug Discovery Research

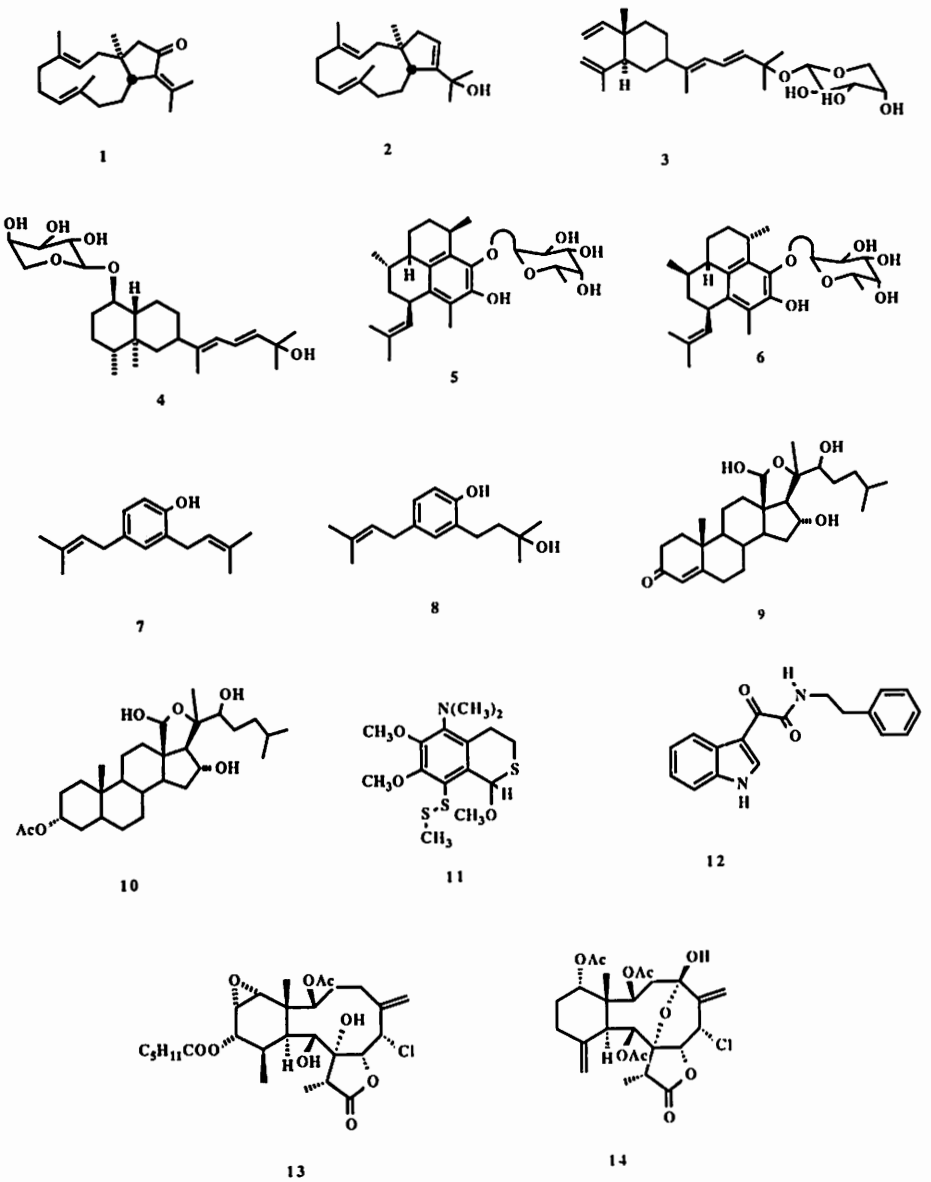
As part of our program to discover new leads, we have continued to collaborate with Jacobs' group in the operation of the University of Miami research vessel *Columbus Iselin*. During the summers of 1988 and 1989, we performed on-board collaborative research consisting of collections, integrated chemistry, and biological assays. These studies have been enormously productive in identifying new sources for compounds with unique properties. From a Caribbean gorgonian of the genus *Eunicea*, we have isolated two dolabellane diterpenoids, **1** and **2**, that possess antiinflammatory properties. The diterpenoids inhibit the liberation of leukotriene B_4 from human polymorphonuclear (PMN) leukocytes. This unique observation may indicate that the compounds are 5- or 12-lipoxygenase inhibitors. From *Eunicea fusca*, we have also isolated two new glycosides, **3** and **4**, each of which possess arabinose sugars; compound **3** is the arabinoside of fuscol and **4** is an arabinose pyranoside of a new class of diterpenoid molecules. Curiously, only fuscol arabinoside (**3**) possesses antiinflammatory properties. These latter molecules resemble the pseudopterosins in that they are pentose glycosides. There can be little mechanistic overlap, however, as the aglycones are of completely different classes.

We have continued to explore *Pseudopterogorgia elisabethae* for new additions to the pseudopterosin class of antiinflammatory agents. This last year, we discovered two new pseudopterosins, PsK and PsL (**5** and **6**). These new compounds are as potent as and are more available than PsE. The compounds are formally modifications of PsE and are created by placing the fucose at the C-9 hydroxyl position (PsK) and by epimerization at the benzylic position of the aglycone (PsL).

Land-based expeditions to the Philippines and to western Australia were also productive. We isolated the new alkylated phenol derivatives **7** and **8** from the brown alga *Encyothalia floculossa* and the cyclic steroidal hemiacetals **9** and **10** from the gorgonian *Ctenocella labiata*. These new compounds are currently under biomedical study in the Jacobs' laboratory. Studies of new organisms collected in the Philippines have been exceptionally rewarding. From a collection of the solitary ascidian *Polycarpa auzata*, we have isolated some structurally unusual, highly antifungal sulfur compounds characterized by polycarpamine A (**11**). These compounds are very potent inhibitors of the human pathogen *Candida albicans* and represent a completely new class of antifungal agents. From another ascidian, we have isolated a new class of indole alkaloids characterized by **12**. Compound **12** controls inflammation in the mouse ear assay, and is an inhibitor of phospholipase A2 in *in vitro* enzyme testing. As part of these Indo-Pacific explorations, we have completed studies of the solenolides (**13**) and the junceellolides (**14**), briarane diterpenoids from Pacific gorgonians of the genera *Solenopodium* and



catechol o-quinone adduct



Junceella, respectively. Although the briaranes have been known since 1975, their potent antiviral, antitumor, and antiinflammatory properties were overlooked previously. The briarane diterpenoids afford important opportunities for drug development, and we will continue to biotest and purify derivatives from this important class. Our aspirations include evaluating these compounds against the human immunodeficiency virus (HIV), the causative virus of acquired immunodeficiency syndrome (AIDS).

Developmental Research with the Pseudopterosins

Over the past year, we have continued to interact with the Jacobs' group to foster the commercial development of the pseudopterosin class of antiinflammatory agents. Our major goal was to determine the pharmacological mechanism of action of this class of compounds. Since the commercial development of these compounds depends on knowledge of how they act to control inflammation, we have made this effort a major component of our studies. We have worked closely with graduate student Ed Luedke to perform various chemical and pharmacological experiments to solve this problem. At this point, we believe the pseudopterosins (exemplified by PsE) act by the direct inhibition of the arachidonic acid-liberating enzyme phospholipase A_2 (PLA_2). This conclusion is based on a series of experiments using inflammatory cells, PMN leukocytes and macrophages, and isolated arachidonic acid-pathway enzymes. Compounds that inhibit PLA_2 represent a new approach to the development of antiinflammatory drugs. The only other authentic PLA_2 inhibitor known is our sponge metabolite manoalide, which has been advanced in commercial development to the clinical trial stage.

We have also collaborated with the Jacobs' group to define the chemical behavior of PsE that is responsible for the observed potent PLA_2 binding. Our current proposal for the mechanism is that the fucose component of PsE imparts selective cell binding to PMN leukocytes. Once surface-selected, PsE is absorbed and then hydrolyzed with intracellular fuscosidases to liberate the intact aglycone. Oxidases then convert the catechol to the orthoquinone, which is the reactive component of PsE. The quinone has already been shown to possess potent PLA_2 binding properties in *in vitro* testing.

Commercial Development of Antiinflammatory Agents

We have dedicated considerable efforts toward the introduction of our program to numerous pharmaceutical industries. The pseudopterosins continue to be at the forefront of our research and of great interest to our industrial collaborators. The productivity of our program has stimulated several pharmaceutical companies to establish programs in marine natural products chemistry.

Cooperating Organizations

Merck Sharpe and Dohme Company
Schering Plough Corporation
Sterling Drug Company

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Fenical, W. The ocean, a sea of chemicals. Presented at San Gorgonio Section, American Chemical Society, University of California, Riverside, May 29, 1986.
Fenical, W. Marine natural products as exploratory leads in the development of anti-inflammatory drugs. Presented at University of California, Los Angeles, Organic Chemistry Colloquium, June 12, 1986.
Fenical, W. The ocean, a sea of chemicals. Presented at Distinguished lecture series, Philip Morris Company, October 22, 1986.
Fenical, W. The ocean, a sea of chemicals. Presented at San Diego Section, American Chemical Society, October 28, 1986.
Fenical, W. New marine natural products: Structure and function. Presented at University of California, San Diego, Chemistry Department, December 1, 1986.
Fenical, W. Soft-corals, a rational source for novel anti-inflammatory drugs. Symposium speaker, American Society of Pharmacognosy, University of Rhode Island, July 22, 1987.
Fenical, W. Soft-corals, rational resources for the isolation of anti-inflammatory analgesics. Invited lecture, School of Pharmacy, Oregon State University, October 21, 1987
Fenical, W. Marine natural products, a unique resource for biologically active compounds. Department of Chemistry Seminar, San Diego State University, April 1987.
Fenical, W. Symbiotic corals, rational sources for the development of anti-inflammatory drugs? U.S.-Japan Cooperative Cancer Research Program, University of Hawaii, February 1987.
Fenical, W. Anti-inflammatory leads from marine soft corals. Presented at Schering Plough Research Laboratories, Malvern, Pennsylvania, May 5, 1988.
Fenical, W. Anti-inflammatory compounds from marine sources. Presented at Department of Chemistry Seminar, University of California, Santa Cruz, April 14, 1988.
Fenical, W. Marine natural products as novel resources for inhibitors of prostaglandin biosynthesis. Presented at College of Pharmacy, University of Utah, March 26, 1988.
Fenical, W. The biomedical potential of Caribbean-based gorgonian corals. Plenary lecture, Mini-symposium on Marine Natural Products Chemistry, University of Puerto Rico, December 1988.
Fenical, W. The natural halogen cycle. Presented at Special Symposium, Department of Biological Sciences, University of California, Santa Barbara, June 5, 1988.
Fenical, W. Recent frontiers in marine natural products chemistry. Presented at Gordon Research Conference on Natural Products Chemistry, New Hampton, New Hampshire, July 1989.
Fenical, W. Marine natural products, a biomedical frontier. Presented at Special Seminar, Sterling Drug Company, February 15, 1989.
Fenical, W. Development of anti-inflammatory drugs from marine algae. Presented at International Seaweed Symposium, Vancouver, B.C., Canada, August 25, 1989.

Marine Chemistry and Pharmacology Program: Development of New Pharmaceutical Agents from Marine Invertebrates

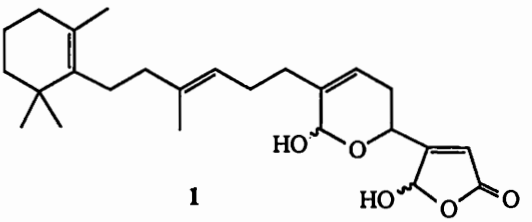
Scripps Institution of Oceanography, UCSD
R/MP-40
Project Initiated: October 1, 1986
Project Completed: September 30, 1989

D. John Faulkner

Throughout the past 3 years we have made steady progress toward our goal of identifying new metabolites from marine invertebrates, which have useful pharmacological properties. A few of these lead compounds are being considered for pharmaceutical development, and one series of compounds related to the lead compound manoalide is in clinical trials. This progress has been accomplished with some difficulty because the combination of constant funding (for which we are very grateful) and rapidly rising costs (particularly overhead) has resulted in a severe cut in the level of effort applied to this project. The most serious impact has been in the discovery phase of the research, which is critically dependent on a generously funded collection program.

The clinical development of manoalide (**1**) is being vigorously pursued by Allergan Pharmaceuticals in (Irvine, California). Allergan has established a program to synthesize analogues of manoalide and has discovered that some simple analogues are almost as active as the lead compound. These discoveries may result in the replacement of manoalide as the commercial drug, but replacement of natural products by analogues is a normal event. Inasmuch as manoalide has guided researchers toward new pharmaceutical products, the collaborative project has been completely successful. From an academic point of view, uncertainty about the mechanism of action of manoalide provides the impetus for further research. During the past 3 years, we have isolated a number of naturally occurring analogues of manoalide (Kernan et al., 1987; Albizati et al., 1987; Kernan and Faulkner, 1988b; Kernan et al., 1989). We have also developed a new procedure for the partial synthesis of manoalide analogues by regiospecific oxidation of naturally occurring furans (Kernan and Faulkner, 1988a). Fifteen of these compounds were used by Dr. Jacobs' group in a structure-activity study to determine which functional groups of manoalide were required for binding with phospholipase A_2 (PLA_2) (Glaser et al., in press). Further research in this area is required to elucidate the mechanism of action of manoalide on (PLA_2).

During the reporting period, we have supplied 65 new marine natural products for screening by Dr. Jacobs'



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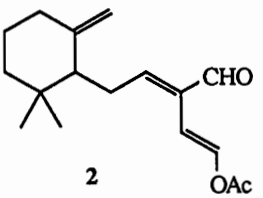

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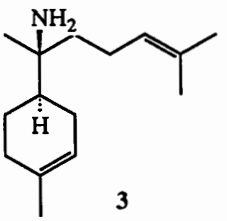

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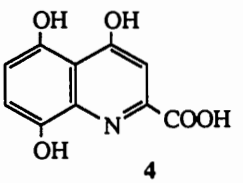

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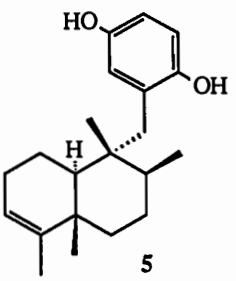

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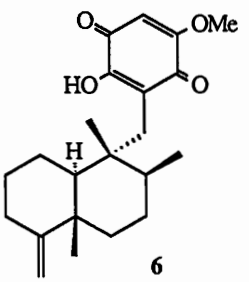

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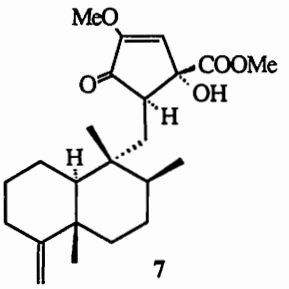

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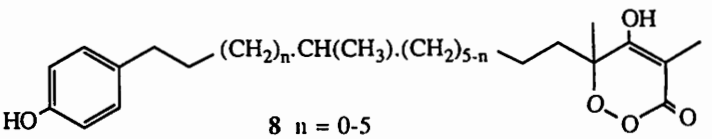

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group. In addition, we have resupplied a number of compounds that were required for a new structure-activity study. Some of these compounds were obtained by re-collection and isolation, but an increasing number were prepared by simple (one- or two-step) chemical modification of relatively abundant compounds. The only disappointment in this area has been our inability to obtain additional samples of some promising compounds from India. We have provided a sample of onchidal (**2**) to Dr. Abramson (Department of Pharmacology, University of California, San Diego [UCSD]) for mechanistic studies that have led to the discovery of a new class of acetylcholinesterase inhibitors (Abramson et al., 1989).

The following chemical studies have been completed:

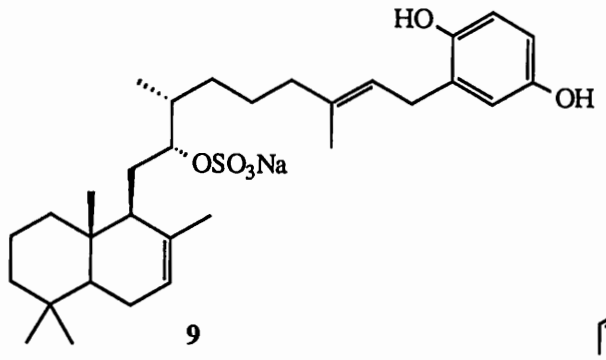
1. The antimicrobial constituent of a sponge of the genus *Halichondria* was found to be the simple sesquiterpene amine, (6R,7S)-7-amino-7,8-dihydro-α-bisabolene (**3**) (Sullivan et al., 1986).

2. Two novel diterpenes and an antimicrobial quinoline derivative (**4**) were isolated from the Antarctic sponge *Dendrilla membranosa* (Molinski and Faulkner, 1986, 1988).

3. A report by Sarin et al. (1987) that avarol (**5**) and the corresponding quinone "may prove to be useful in the treatment of patients with AIDS and AIDS-related complex" led us to isolate a number of naturally occurring quinones for screening. In the process of reisolating ilimaquinone (**6**), four new metabolites, exemplified by dactylospongenone A (**7**), were isolated from a Palauan species of *Dactylospongia* (Kushlan et al., 1989). However, the anti-AIDS activity of avarone could not be confirmed by Dr. Spector, Department of Pediatrics.

4. During a related study to isolate the antiviral constituent of a sponge of the family Plakinidae, the antiviral compound decomposed, but an interesting new perlactone (**8**) was identified.

5. The halisulfates are a group of six sulfated terpenoids from a La Jolla sponge of the family Halichondriidae (Kernan and Faulkner, 1988b). Halisulfates 1 (**9**) and 2 (**10**) both showed good anti-inflammatory activity in screens performed by Jacobs and coworkers.



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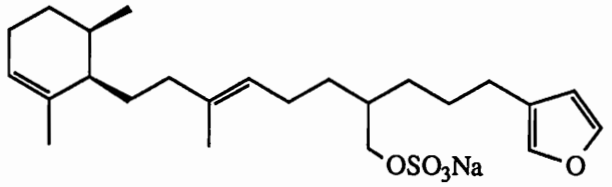

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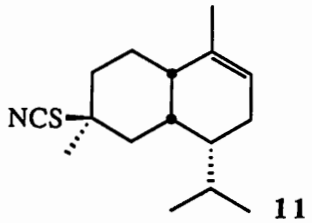

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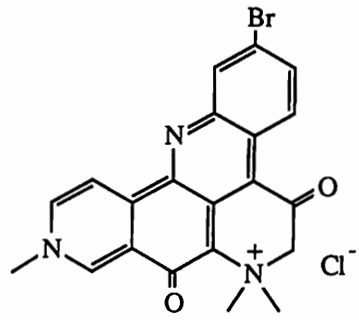

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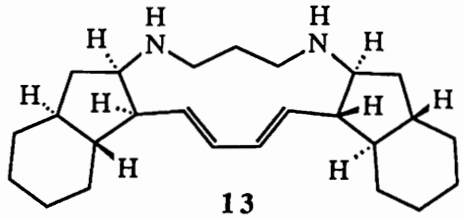

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6. A group of sponges containing isonitriles or isothiocynates have been studied extensively. Three new diterpene isonitriles were found in a Palauan species of *Halichondria* (Molinski et al., 1987). The sponge *Trachyopsis aplysinoides* contains the sesquiterpene thiocyanate (**11**), the first example of this functional group in a marine natural product, and three sesquiterpene isothiocyanates (He et al., 1989). Additional sesquiterpene isothiocyanates have been found in an *Axinyssa* species (Marcus et al., 1989), and very recently, a second thiocyanate has been discovered.

7. The antimicrobial activity of many sponges has been traced to alkaloids. These studies have resulted in the isolation of petrosamine (**12**) from a species of *Petrosia* (Molinski et al., 1988) and halichlonadiamine (**13**) from a species of *Haliclona* (Fahy et al., 1988).

8. A number of diterpenes of the "spongian" class have been isolated from dendroceratid sponges and the nudibranchs that feed on them. Bobzin is testing the hypothesis that the most biologically active sponge metabolites are those that are selectively concentrated in nudibranchs (Bobzin and Faulkner, 1989a, 1989b; see also Kernan et al., 1988).

9. We collaborated with Dr. Crew's group to revise the structure of suvanine (Manes et al., 1988).

10. Our collaboration with

Professor C. B. Rao (Andhra University, India) has resulted in the discovery of a new series of anti-inflammatory alkaloids from a species of *Zoanthus* (Rao et al., 1989a). We have also identified new metabolites from the gorgonian *Isis hippuris* (Rao et al., 1988) and the sea hare *Aplysia dactylomela* (Rao et al., 1989b).

The majority of the new compounds described above have been studied by Dr. Jacobs and his group at the University of California, Santa Barbara, and their results are reported in the "Annual Status Reports" of that institution.

Cooperating Organizations

Allergan Pharmaceuticals
SKF Laboratories
Wyeth-Ayerst Laboratories

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Marine Natural Products in Pharmacology: Development of Leads from Marine Animals

University of California, Santa Cruz
R/MP-41
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Phillip Crews

Progress in disease chemotherapy research depends on developing new types of organic substructures with biological activity. We wish to contribute to the process of discovering new pharmacological model compounds by focusing on the novel chemistry that is being discovered from marine organisms. Indications that this is a promising approach abound. For example, didemnin B, a cyclodepsipeptide from a tunicate (Rinehart et al., 1981), has joined the ranks of compounds in clinical trial for cancer chemotherapy (Suffness and Douros, 1982). Another series of compounds, the bryostatins, which are macrocyclic polyoxygenated metabolites from bryozoans, have shown very potent activity against several key National Cancer Institute experimental tumor systems (Pettit et al., 1983, 1984). A clinical trial will soon begin in Europe with selected bryostatins, and the National Institutes of Health is undertaking large-scale synthesis of bryostatin-1 for preclinical evaluation against fast- and slow-growing tumors. Manoalide, a sponge sesterpene, has antiinflammatory activity (de Silva and Scheuer, 1980; de Freitas et al., 1984). The bengamides, a series of novel ketide-amino acids that we have isolated (Adamczeski et al., 1989), are being developed by Syntex as new antivirals and anthelmintics. Other recent advances have been summarized in the popular literature, and, as just one example, when Smith (1987) wrote about the search for new pharmaceutical compounds in the oceans, she considered the question: What is the biomedical importance of marine organisms?

Examples in the literature clearly show the novelty of marine natural products chemistry (e.g., Crews and Naylor, 1985). Alongside this are interesting pharmacological observations, for example, observations that extracts from marine organisms have bioactivity in a variety of assays (e.g., McConnell and Fenical, 1979; Fuhrman, 1981; Durros and Suffness, 1980; Rinehart et al., 1981) and that the highest percentage of bioactive marine invertebrate extracts come from coral reef organisms (Green, 1977; Bakus, 1981). Our continuing goal has been to identify new relationships between chemical structure and bioactivity.

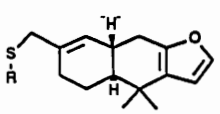
Our aim during the past years has been to continue development of novel marine animal natural products

Table 1. UCSC Sponge Natural Products with Potential Against Infectious Disease: Recent Anti-AIDS *in vivo* Screen Results

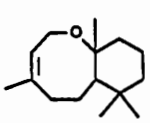
Extract code	Organism	RT activity reduction (%)	HIV Alex Cell (IC_{50} µg/mL)	Alex Cell only (IC_{50} µg/mL)
86079	unknown sponge	56	not active	not toxic
87010	unknown sponge	97	not active	not toxic
87013	Hydroid	94	not active	not toxic
87014	*Astroplakina*	44	not active	not toxic
87043	*C. lochi*	00	0.16	not toxic
87057	*Fascaplysinopsis*	100	0.4	6.2
87063	*Nemidocarpa*	99	not active	not toxic
87067	*Ircinia*?	00	16.2	~55.0
87069	*Callyspongia*	70	not active	not toxic
87077	*Psamaplysilla*?	93	not active	not tested
87079	unknown sponge	56	5.0	in test
87085	unknown tunicate	00	23.0	not toxic
87104	unknown sponge	67	14.0	in test
87111	unknown sponge	82	not active	not toxic
87113	unknown sponge	54	not active	not toxic
87126	*Haliclona*	99	not active	2.2
87129	*Callyspongia*	99	not active	not toxic
87150	*Haliclona*?	98	not active	in test
88003	unknown sponge	92	not active	in test
88013	*Dysidea*?	86	not active	not toxic
88106γ	=87057	70	12.5	67.0

name of the compound family
Genus species
new or old (numbers of compounds)
bioactivity

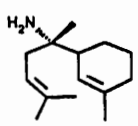
TERPENE DERIVATIVES



(-) thiofurodysinin
Dysidea herbacea
new enantiomer series 1988
antiparasite: *in vitro* [NB,HC]
FIVE active lead compounds
Pending US Patent Application



oxobisabolane
Dysidea herbacea
new, 1989 TWO compounds
antiparasite

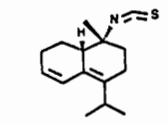


monocyclofarnesanes
unidentified sponge
old, TWO compounds
antiparasite, antimicrob

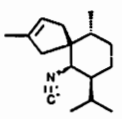
with promise in several relevant pharmacological systems. The emphasis is to extend anti-infectious disease leads identified during our previous project R/MP-33.

Important lead compounds formed the principal basis of our interaction with the collaborators listed at the end of this report. The organisms that provided the active substances were collected from the tropical Indo-Pacific area, and the active compounds were obtained via bioassay-guided isolation. In several cases, follow-up work was initiated, including recollection of active organisms in support of scale-up isolations. A large effort is represented here: More than 40 crude extracts have been evaluated between 1986 and 1989.

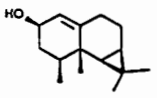
The compounds that continue to be of highest priority for further development are as follows: (1) Bengazole A and B, novel oxazole-containing heterocycles from an undescribed Jaspadae, with anthelmintic properties as described in our approved U.S. patent #4785012; (2) Bengamides A and B, large-ring heterocycles from an undescribed Jaspadae, with anthelmintic properties as outlined in our approved U.S. patent #4,831,135; (3) thiofurodysinin acetate, thiofurodysinin disulfide, and related compounds from *Dysidea herbacea* with potent *in vivo* activity against several helminths, as will be described in a pending patent application USSN 263,261; (4) Plakinidines A and B, unusual purple alkaloids from a *Plakortis* sp. with activity against helminths and the reverse transcriptase enzyme. These are the subject of a pending U.S. patent application, UC Case No. 89-024-1; (5) Prefascapins, unusual aromatic alkaloids from *Fascaplysinopsis* sp. with activity against helminths and the reverse transcriptase enzyme (the subject of a future U.S. patent application); and (6) a series of aminoimidazoles being pursued from Indo-Pacific nudibranchs and sponges that exhibit interesting antiparasite activity (a structure activity study has commenced and is being aided by the evaluation of synthetic compounds that contain a portion of the main pharmacophore), also the subject of a future U.S. patent application.



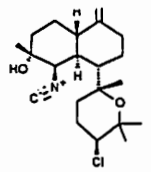
cadelanes
Leucophloeus (= Axinyssa) fenstratus
old, 1988 FOUR compounds
antiparasite, antimicrobial



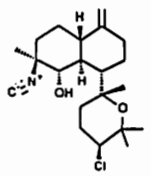
axisonitrile
Topsentia sp.
old, 1988
antimicrob, antiparasite



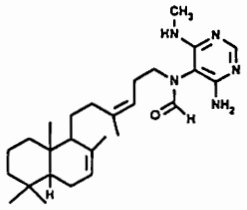
tricyclic sesquiterp
unidentified sponge
new, 1989 TWO compounds
antimicrob, antiparasite



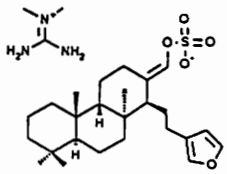
kalihinols
Acanthella carvenosa
old, 1988 FIVE compound
antimicrob, antiparasite



isokalihinols
Acanthella carvenosa
new, 1988 FIVE compounds
antiparasite

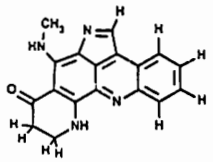


midpacifamide derivatives
Agelas sp.
old, 1989 THREE compounds
antimicrobial

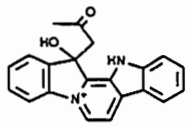


suvanine
Coscinoderma matthewsi
yes 1985, 1988
smooth muscle: ACH

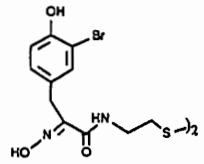
AMINO ACID DERIVATIVES



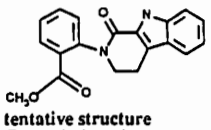
plakinidines
Plakortis sp.
yes, 1989 TWO compounds
anti-RT, antiparasite



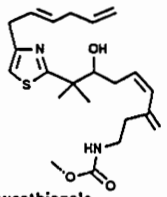
prefascapin
Fascaplysinopsis sp.
yes, 1989 SIX compounds
anti-AIDS: RT, no HIV; antimicrobial



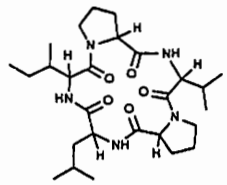
psammaplin
Psammaplysilla sp
yes 1987 SIX compounds
antimicrobial:



tentative structure
Fascaplysinopsis sp.
yes, 1989
antimicrobial



mycothiazole
Spongia mycofijiensis
yes, 1988
antimicrobial:, antiparasite



fenestins
Leucophloeus (= Axinyssa) fenstratus
new, 1988 TWO compounds
antimicrobial

Specific structural and status information for new compounds and promising extracts are summarized in Tables 1 and 2.

Cooperating Organizations
National Cancer Institute
National Institutes of Health
SeaPharm, Ft. Pierce, Florida
Syntex Research, Inc, Palo Alto, California
University of California Research Expeditions Program

Collaborating Scientists
Dr. Tom Matthews, Institute of Antimocrobial & Antiviral Chemotherapy, Syntex Research
Dr. Oliver McConnell, SeaPharm Project of Harbor Branch
Dr. Matthew Suffness, Natural Products Branch, National Cancer Institute

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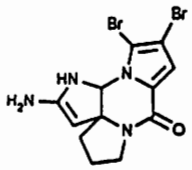
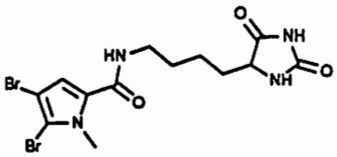
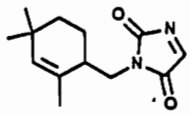
ALKALOIDS



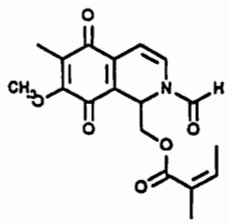
dibromophakellin
Agelas sp.
no, 1989 TWO compounds
antimicrobial



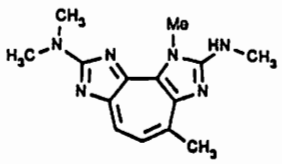
no name
Agelas sp.
known series
antimicrobial



tentative structure
Dysidea sp.
new, 1989 FIVE compound
antimicrobial

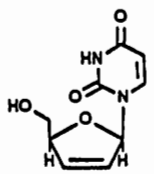


quinones
unidentified sponge
known compound series
antimicrobial



bis imidazoles
unidentified hydroid
new, 1989 TWO compounds
antimicrobial

NUCLEOSIDES



DDDU
Geodia neptuni
known series, TWO compounds
antimicrobial

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Table 2. UCSC Sponge Natural Products with Potential Against Infectious Disease: Recent Antihelminth *in vivo* Prescreen Results

Extract code	Organism	% Reduction vs control against *N. brasiliensis* at 50 µg/mL CSTS	VIAB	MOT
86008	*Acanthella*	98	98	50
86009αF1	*Jaspis*	82	92	50
86012	unknown sponge	39	26	25
86014	unknown sponge	47	79	25
86017	*Dysidea*	46	26	25
86018	unknown sponge	19	65	25
86031	unknown sponge	52	72	50
86032	unknown sponge	71	96	75
86037	*Spongia*	74	89	50
86039	unknown sponge	78	00	00
86043	unknown sponge	46	00	50
86046	*Acanthella*	100	100	100
86049	unknown sponge	94	48	50
86051	unknown sponge	45	00	00
86053	unknown sponge	40	00	00
86056	unknown sponge	41	00	00
86059	unknown sponge	43	00	00
86061	unknown sponge	71	45	00
86064	unknown sponge	48	00	00
86069	*Dysidea*	63	25	00
86070	*Dysidea*	86	96	100
86073	unknown sponge	40	00	00
86074	*Psammaplysilla*?	45	00	00
86087	*Haliclona*?	10	38	50
87011	*Jaspis*	92	24	50
87014	?*Astroplakina*	90	85	80
87016	unknown sponge	55	30	00
87025	unknown sponge	60	00	00
87028	unknown sponge	46	03	25
87031	unknown sponge	72	49	50
87036	*Jaspis*	83	18	25
87043	*C. lochi*	21	07	00
87057	unknown sponge	10	07	25
87058	=86061?	91	100	100
87089	unknown sponge	29	08	00
87126	*Haliclona*	22	64	50
87126γ	*Haliclona*	90	100	100
87150	unknown sponge	41	53	25
88005	unknown sponge	43	05	00
88010	*Ircinia*?	19	02	00
88015	*Acanthella*	25	100	100
88017	*Leucocphloeus*	22	55	50

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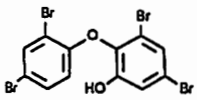
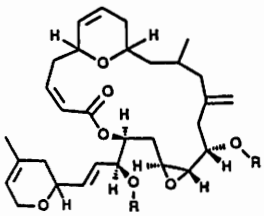
SHIKIMATE DERIVATIVES

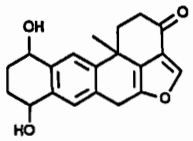


polyhalo phenol ethers
Carteriospongia & Dysidea
known, FIVE compounds
antimicrobial

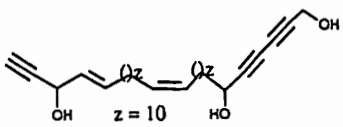
POLYKETIDE DERIVATIVES



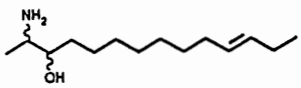
fijianolides
Spongia mycofijiensis
new, 1988 TWO compounds
Colon, antiparasite



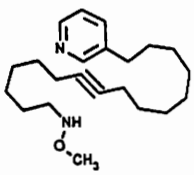
haliquinol
Reniera sp.
known series, FOUR compounds
antimicrobial



melyne
Xestospongia sp.
new, 1987 THREE compounds
Giardia



halamineols
Xestospongis sp.
known, 1988 THREE compounds
antiparasite, antimicrobial



niphatyne
Niphates sp.
yes, 1987 THREE compounds
P388

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Effect of a Marine Algal Constituent on the Growth of Lettuce and Rice Seedlings

University of California, Berkeley
R/MP-43
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Isao Kubo, et al.

Since the era of the Roman empire, seaweeds have been reported as a source of an agricultural fertilizer, and seaweeds are still used today in some areas of the world for this purpose. While some of their utility is no doubt due to organic nutrients and inorganic minerals, algal constituents have been isolated that exhibit specific control over terrestrial plant growth. Both gibberellins and cytokinins have been isolated from algal sources. During our continuing search for natural products as a resource for biologically active compounds, we examined the extracts of the red algae *O. washingtoniensis* Kylin and *O. floccosa* (Esper) Falk (Rhodophyta) for the presence of any phytoactive components. Preliminary bioassays using lettuce and rice plants revealed that the MeOH extract of *O. washingtoniensis* and *O. floccosa* possessed potent growth-stimulatory activity. We report the isolation, identification, and biological activity of α-O-methyllanosol(3,4-dibromo-5-methoxy-methyl-1,2-benzenediol) [**1**].

Isolation and Identification of a Plant Growth Stimulator

The MeOH and/or the Ch_2Cl_2 extracts from the algae gave the biologically active compound, which was recrystallized from C_6H_6 as colorless needles (melting point, 130°C). The mass spectrum indicated it to be a dibromo compound $C_8H_8O_3Br_2$ ([*M*]$^+$ at 310, 312, 314, (1:2:1)). The ^{13}C nuclear magnetic resonance (NMR) spectrum indicated a penta-substituted benzene with absorptions at δ 145.1 (s), 143.8 (s), 129.2 (s), 114.6 (d), 113.5 (s) and 113.0 (s), a methoxy group at δ 57.7 (q), and a benzylic methylene group at δ 74.0 ppm (t). The ^1H NMR spectrum showed the signals for an aromatic proton at δ 6.95 (s, 1H); a methoxy group at δ 3.32 (s, 3H); and a benzylic methylene group at δ 4.38 ppm (s, 2H), respectively. The 10.72% nuclear Overhauser effect (n.O.e.) of the aromatic proton, observed when the benzylic methylene group was irradiated at 4.38 ppm, indicated a close spatial relationship between these two groups. In spite of the possible proton exchange with the solvent CD_3OD, the two signals from the two hydroxy protons were observed at δ 10.1 (s) and δ 9.58 ppm (s). This implies a 1,2-relationship of the two hydroxy groups, with a strong intramolecular hydrogen bond keeping the protons from exchanging with the solvent. From these spectra, this compound was identified as α-O-methyllanosol, a compound previously isolated from other red algae (e.g., Odonthaliaceae [Katsui et al., 1967; Pederson et al., 1974]; Polysiphoniaceae [Fenical, 1975]; Rhodomelaceae [Katsui et al., 1967; Pederson et al., 1974; Kurata and Amiya, 1977]).

The 3,4-dibromo-5-methoxymethyl-1,2-benzenediol [**1**] was previously synthesized by Matsumoto and Kagawa (1964); however, the experimental details were not reported. The interesting biological activities of [**1**] on plant growth prompted a more efficient synthetic method for the preparation of [**1**] and its derivatives.

Synthesis of [1]

An attempt to brominate 5-bromo-4-hydroxy-3-methoxy-benzaldehyde (Raiford and Perry, 1942) [**2**] with 2.1 eq of Br_2, refluxing AcOH and using iron powder as a catalyst, gave 5,6-dibromo-4-hydroxy-3-methoxybenzaldehyde [**3**] in a low yield of 27% (see Figure 1). Neither a large excess of Br_2 nor an extended reaction time improved the yield. While the yield of [**3**] was low, it was seen as an improvement over the conventional reaction sequence of methylation, nitration, and diazotization that ends with the Sandmeyer reaction. The structure of [**3**] was confirmed in part by the observation of an n.0.e. enhancement of the C-6 aromatic proton signal (δ 7.44 ppm, 18.4%) when the methoxy methyl proton (δ 4.00 ppm) was irradiated. The ^1H NMR spectrum of [**3**] showed the characteristic signals at δ 4.00 (OMe), 6.12 (OH), and 10.22 ppm (CHO) in agreement with the proposed structure. The infrared

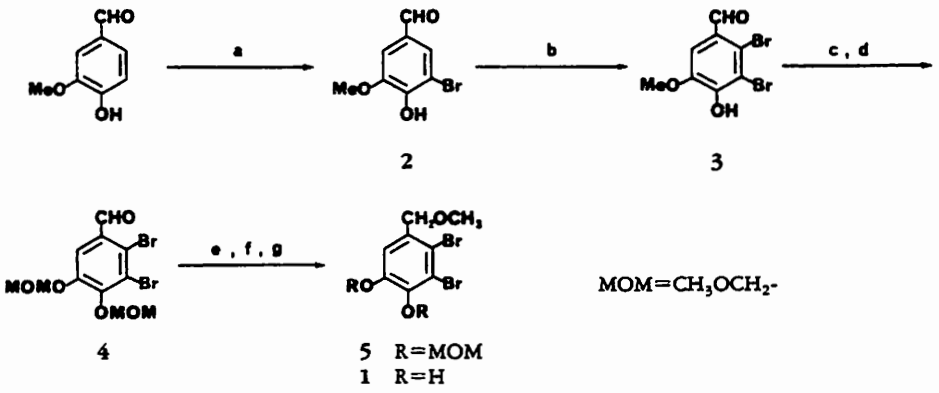

Figure 1. Scheme of the synthesis of **1**. (a) Br_2 (1 equiv)/HOAc/reflux; (b) BR_2 (2.1 equiv)/HOAc/Fe/reflux; (c) BBr_3/Ch_2Cl_2/–50° to ambient temperature; (d) $MeOCH_2Cl$/NaH/THF/0°; (e) $NaBH_4$/EtOH/0°; (f) MeI/NaH/THF/0° to ambient temperature; (g) concedntrated HCl/MeOH/ambient temperature.

spectrum confirmed the presence of a hydroxyl group (3,200 cm^{-1}). Demethylation of [3] with BBr_3 gave an unstable catechol, which on immediate reaction with $MeOCH_2Cl$ and NaH, gave a protected aldehyde [4]. Reduction of [4] with $NaBH_4$ followed by etherification with MeI and NaH gave [5], which—when hydrolyzed with acid—gave [1]. The synthetic product [1] was shown to be identical with the natural compound by direct comparison of physical and spectral properties. Several derivatives of [1] were also synthesized, according to the scheme shown in Figure 2, in order to further investigate their potential for similar biological activity.

Growth-Stimulating Effect of α-O-Methyllanosol on Terrestrial Plants

The presence of α-O-methyllanosol was observed to have little effect on rice seedlings even at the concentration of 100 ppm. However, as shown in Figure 3, the growth of lettuce hypocotyl and root were stimulated at this concentration. The overall appearance of greater health among the seedlings exposed to α-O-methyllanosol was striking: These seedlings were much larger than the seedlings in the control group, and they appeared to be normal in all proportions. No inappropriate elongation was apparent in either the hypocotyl or the root of the lettuce seedlings. Compared with the control seedling, the hypocotyls of the exposed seedlings were a much richer and deeper green, and the roots had more root hairs.

This is the first report on the effect of α-O-methyllanosol on terrestrial plants, although other related halogenated phenolics have been used as herbicides (Raiford and Witmer, 1945). Lettuce seedlings are inactive to indoleacetic acid (IAA) but active to gibberellins (GAs). Although the structure of α-O-methyllanosol seems to resemble synthetic auxins such as 2,4-D, the results from the growth experiments with lettuce seedlings show GA-like activity. Further work is now in progress to elucidate the mechanism of this growth regulator.

There are several possible explanations for the effects of α-O-methyllanosol on plants; α-O-methyllanosol may be a new class of plant-growth regulators showing GA-like activity. Some phenolic substances may be involved as algal-growth regulators. For example, the free benzyl alcohol lanosol, its sulfonate, and other simple phenols have been observed to both stimulate and depress the growth of marine algae; specifically, Kamisaka (1973) has reported that lower concentrations of lanosol strongly stimulated the growth of red algae. One report (Fries, 1973) suggests that excreted phenolic substances may be necessary for the normal morphology and completion of the life cycle in algal species of *Ulva* and *Monostroma*. Also, these or similar compounds may be used as antifouling agents. Many simple phenols exhibit antibacterial and antifungal activity, and it has been suggested that simple phenols may be involved in regulating the growth of epiphytes and parasites in algae (Provasoli, 1969).

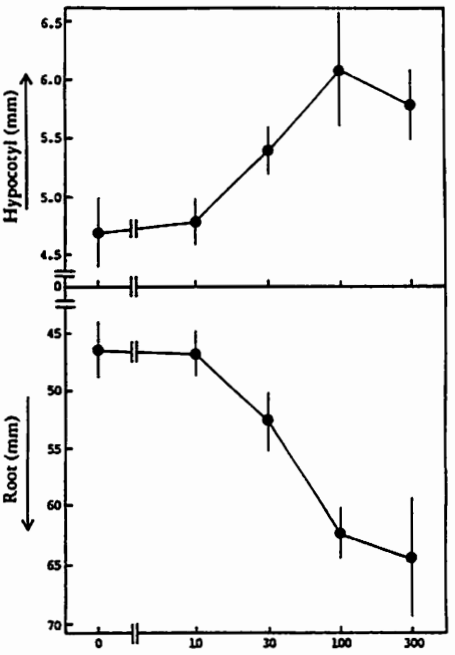

Figure 3. Effect of α-O-methyllanosol [1] on the growth of lettuce seedlings. Vertical lines represent standard error.

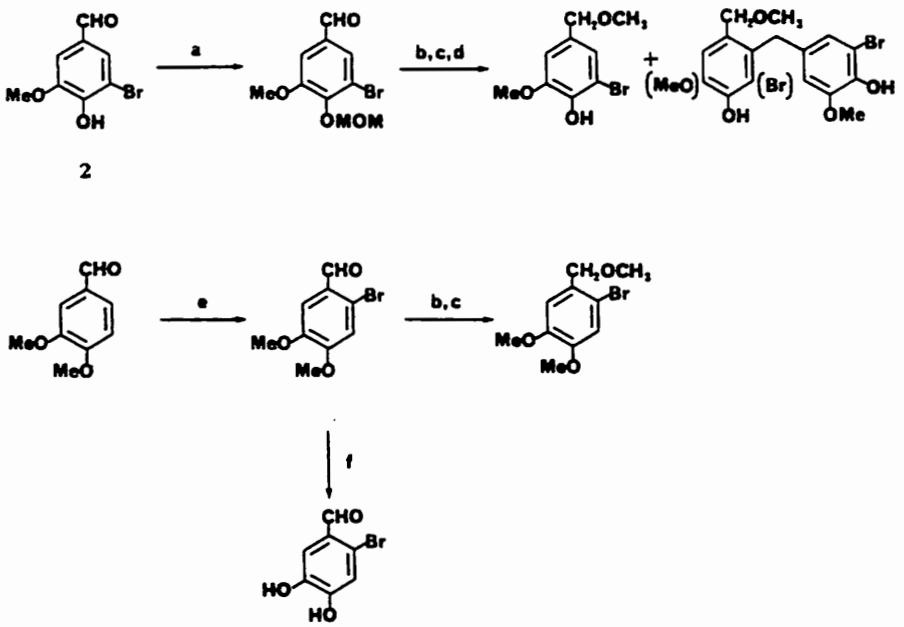

Figure 2. Scheme of the synthesis of several derivatives of 1. (a) $MeOCH_2Cl/NaH/THF/Et_3N$; (b) $NaBH_4/EtOH$; (c) MeI/NaH/THF; (d) concentrated HCl/MeOH; (e) $Br_2/CHCl_3$; (f) BBr_3/CH_2Cl_2.

Cooperating Organizations

FMC
Kinki University, Osaka, Japan
Native Plant Resource Institute
Osaka University, Osaka, Japan
Safer Chemical Corporation
Suntory Institute for Bio-Organic Research
Takasago Institute for Interdisciplinary Science
Zoecon Corporation

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Ocean Engineering

Field Test of Doppler Acoustic Directional Wave Sensor

University of California, San Diego
R/OE-4
Project Initiated: October 1, 1986
Project Completed: September 30, 1989

R. L. Lowe and R. T. Guza

Introduction

Surface gravity waves are the largest single source of energy dissipated on exposed coasts. Accurate measurement of the directional properties of ocean waves are essential for proper design of coastal structures and for estimating longshore sand transport.

A prototype Doppler acoustic instrument for measuring wave directional properties has been developed and field tested. The instrument is compact and shows promise for good directional resolution. Numerical methods appropriate for analysis of data from compact measurement systems have been refined (Herbers and Guza, 1989).

System Description

A prototype Doppler acoustic directional wave sensor (DADS) has been developed and constructed (Figure 1). The unit is mounted near the seabed in water depths up to 15 m. Four pairs (i.e., transmitter and receiver) of 1-MHz transducers are housed in two precision-machined polyvinyl chloride (PVC) bars. These 30-cm-long bars are orthogonally mounted on the top of an aluminum cylinder (diameter, 20 cm) housing the electronics. The PVC bars are machined so that a transducer pair ensonifies a volume centered 1 m (100 cm) from the instrument (Figure 2). A Doppler shift in the received sound is caused by the fluid velocity directed in the plane and along the bisect of the transmitting and receiving transducers (Figure 2). In the present configuration, the beams are oriented 45° from the horizontal plane. The net result is a system (Figure 3) that measures the water velocity at four points on a circle, 1.4 m in diameter and 0.7 m above the top of the instrument. These velocity data contain, in principle, much information about the wave directional spectrum. Instrument power and signals are cabled to a location above water.

The conceptual design is patterned after the proposed continuous wave (CW) system modified to eliminate leakage of the transmitted signal into the receiver through the instrument's mechanical structure. Leakage has been overcome by pulsing the 1-MHz transmitted signal for a duration of 1 ms every 5 ms, with the receiver listening during the off period.

A functional block diagram of the system is shown in Figure 4. Two 1-MHz, square-wave signals, 90° out of phase, are produced from a 4-MHz crystal oscillator. The drive pulse for the transmitting transducers are generated by the zero-phase signal and inherently stable, digital counter circuits. One counter determines the length (1 ms) of the pulse, while the other determines the repetition rate (every 5 ms). The 1-ms pulse is distributed to four identical driver circuits. The transmitter-driver circuits provide the electrical energy to the transducers, which is converted to mechanical (sound) energy. A boostrap circuit using power-MOS-FETs (metal-oxide-semiconductors and field-effect transistor [FETs]) is used. This circuit allows a driving voltage larger than the logic supply voltage. The FET selected (VN2406) has an on-resistance of 6 ohms and can generate pulses of up to 100 v. Little effort was expended optimizing the driver circuit. The main objective was simply to produce enough transmitted sound energy so that the small fraction of backscattered energy was detectable.

Figure 1. Continuous-wave Doppler acoustic instrument. The instrument case is 30 cm tall and 20 cm in diameter.

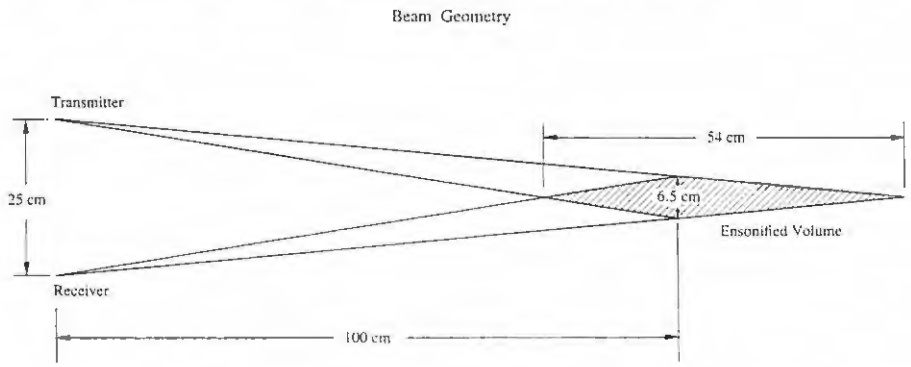


Figure 2. Plan view of the ensonified volume formed by the intersection of the transmitter and receiver beams.

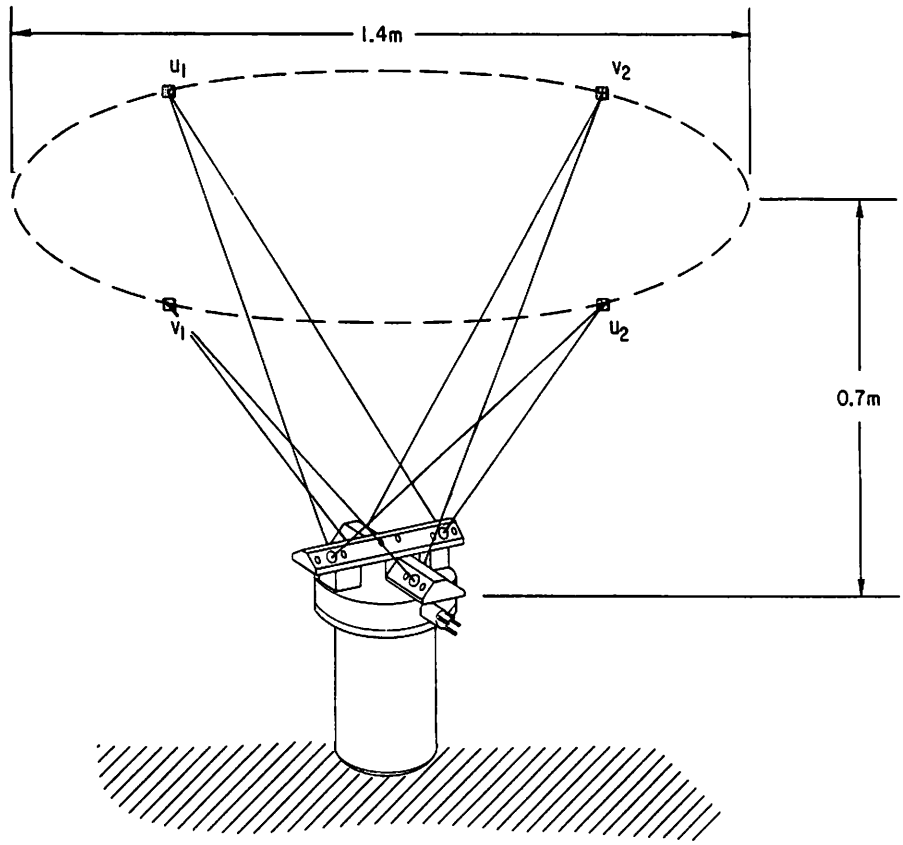


Figure 3. Geometry of the continuous-wave acoustic Doppler instrument showing the points of velocity measurements relative to instrument.

Four identical receivers are used, each measuring the Doppler frequency of the backscattered sound, and hence a fluid velocity component, at different spatial points. The backscattered sound is converted by the receiving transducer to an electrical signal that is amplified and to an impedance matched by a wide-band, fixed-gain, operational amplifier. Next, the signal is further amplified by an intermediate-frequency (IF) amplifier whose gain is automatically controlled by a feedback voltage (AGC) produced by combining the processed signals. The intensity of the received signal is proportional to the vector sum of the Co and Quad signals. AGC is obtained by low-pass-filtering the vector sum of the Co and Quad signals. The AGC loop has a response time about 1 second and a range of 60 dB (10^3 in voltage). The AGC holds the output of the IF amplifier constant over periods that are long relative to the transmitter's repetition rate. Thus, the AGC compensates for variations in the intensity of the backscattered signal.

After the received signal has been amplified to approximately one volt root-mean-square, it is sent to two wideband (10 MHz), full (four quadrant) multiplier circuits. The received signal is multiplied by the locally generated 0°-phase and 90° phase, 1-MHz, square-wave reference signals. Each of the resulting signals is low-pass-filtered by three-pole Bessel filters whose phase characters have been carefully matched. The 3-dB cutoff frequency chosen, 2 kHz, corresponds to a water velocity of approximately 1.5 m/s. The above procedure yields a complex demodulation of the originally received backscattered signal. The coincident and quadrature correlation (Co and Quad) signals for each receiving transducer produced by complex demodulation are sampled by a timing pulse formed by one-shots. This sampling pulse is delayed from the beginning of the transmitted pulse by 1.1 ms and has a duration (approximately 1.3 ms) that encompasses the entire time the sound pulse is passing through the ensonified volume. The sampled Co and Quad signal pairs for each of four transducer pairs are the outputs returned to a small personal computer (PC) located above the water.

The four Co and Quad signal pairs are digitized using a high-speed, analog-to-digital converter (70,000 conversions/s) controlled by the PC. Finally, the Doppler frequencies (thus the fluid velocities) are determined autocovariance (pulse-pair) estimator (Lhermitte, 1985) and stored by the PC.

Two methods of processing the Co and Quad signals have been implemented. An incoherent method uses Co and Quad signals from a signal return (1-ms duration) to determine the Doppler frequency. The incoherent method generally yields unaliased velocity measurements (in our case, the first alias occurs at approximately 2 m/s, a velocity seldom attained), but it suffers noise known as "self clutter" caused by finite returns from nonoverlapping regions of the ensonified volume (Smith, 1989). The theoretical noise level using the parameters for DADS is about 5 $(cm/s)^2$. Assuming this noise to be white, the predicted noise density is 2.5 $(cm/s)^2$ Hz. Smith (1989) points out that this is an optimistic noise estimate but probably is correct within a factor of 2 or 3. For the May data sets (discussed below), the noise spectral density in the frequency above 1 Hz (well above that of surface waves) varied between 5 and 25 $(cm/s)^2$ Hz. The lower noise level is about equal to the estimated self clutter, whereas the higher noise density is probably caused by surface returns (discussed in the next section).

The second method for obtaining the Doppler frequency is known as coherent processing. It relies on the backscattered sound energy being coherent from one transmitted pulse to the next. The individual backscatters are assumed to not rearrange themselves between transmitted pulses. Coherent

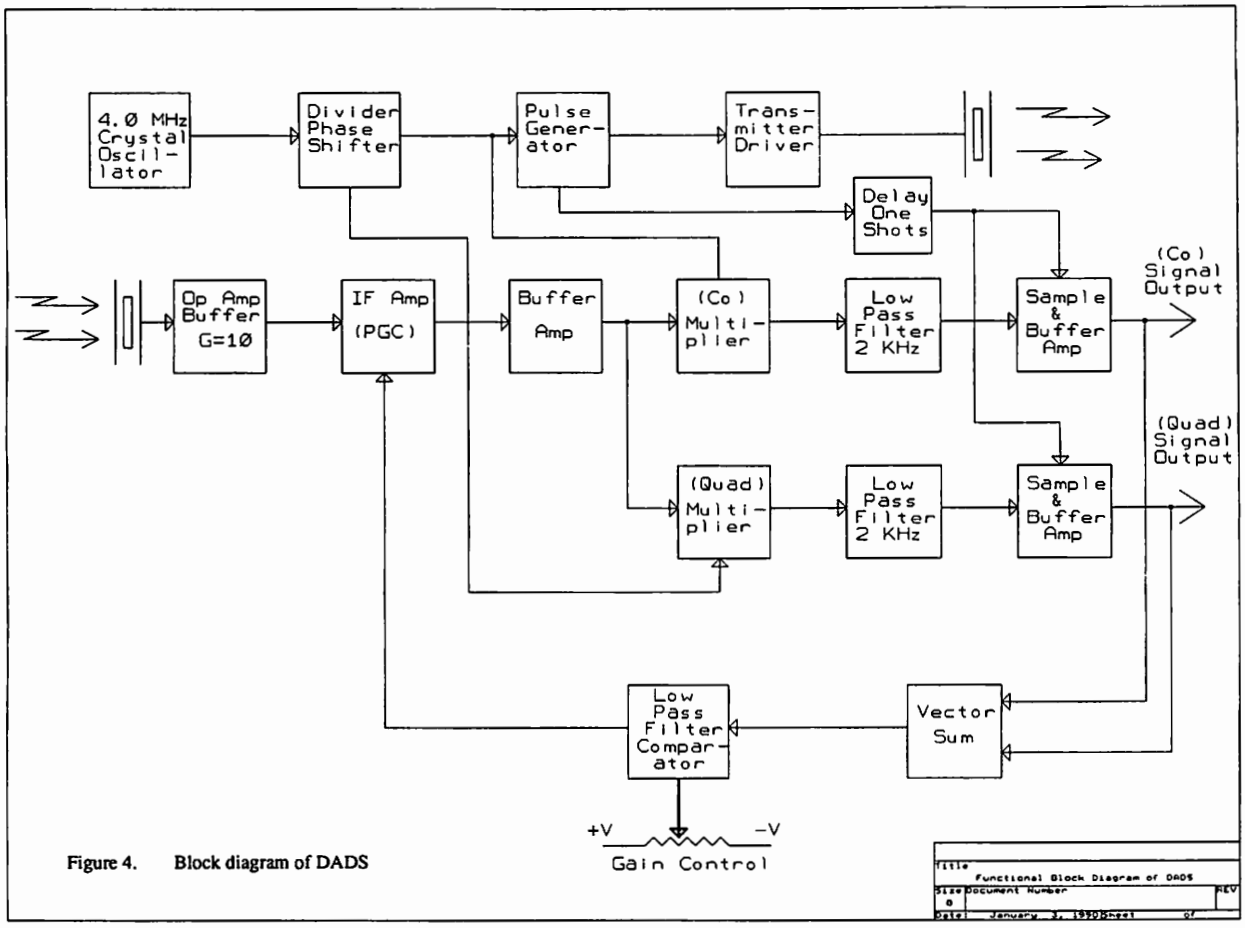


Figure 4. Block diagram of DADS

Figure 4. Block diagram of DADS.

processing eliminates self clutter because exactly the same volume is ensonified with each pulse. The Co and Quad signals are sampled at the rate of the transmitted pulse, 200 Hz, thus causing aliasing to begin at 100 Hz. A 100-Hz Doppler frequency corresponds to a water velocity of only 7.5 cm/s, so typical wave data are highly aliased. A de-aliasing algorithm was implemented. Summarizing, the incoherent method yields unaliased, but noisy, data, while the results from the coherent method are less noisy but aliased.

Surface Reflections

Surface reflections seem to be a problem for the chosen system configuration. With the beams tilted 45° to the water surface, the sidelobes that intersect at the surface are 40 dB (10^4) lower than the main beam. Thus, the surface would have to be about 10^4 times more reflective than the ensonified volume to cause a problem.

To investigate the surface reflection, the transmitted pulse rate was lowered from 200 to 20 pulses/s. At this rate, the sound pulse could travel 75 m between transmitted pulses, and therefore, in 6 m of water, any surface reflection should be clearly seen on an oscilloscope. Surface returns were indeed observed and were as much as five times stronger than the return from the ensonified volume. Acoustic shielding was added to the instrument in an attempt to eliminate the surface interference but could only reduce it by about a factor of four. If the energy in these sidelobes intersects the surface during a time the receiver is on, the resulting Doppler frequency estimates will be seriously contaminated. The performance of DADS appears to be a function of tidal stage, as would be expected if surface reflections are a problem.

When DADS is working properly (not contaminated by surface returns), the resulting velocities appear to be of excellent quality. Figure 5 is a comparison of the cross-shore velocities from two different sonar beams and a co-located pressure sensor. This time series shows clearly that the measured wave-induced velocity is coherent with the bottom pressure, as it should be.

Comparison Experiment

In order to quantitatively evaluate DADS' ability to measure oscillating currents, from which moments of the

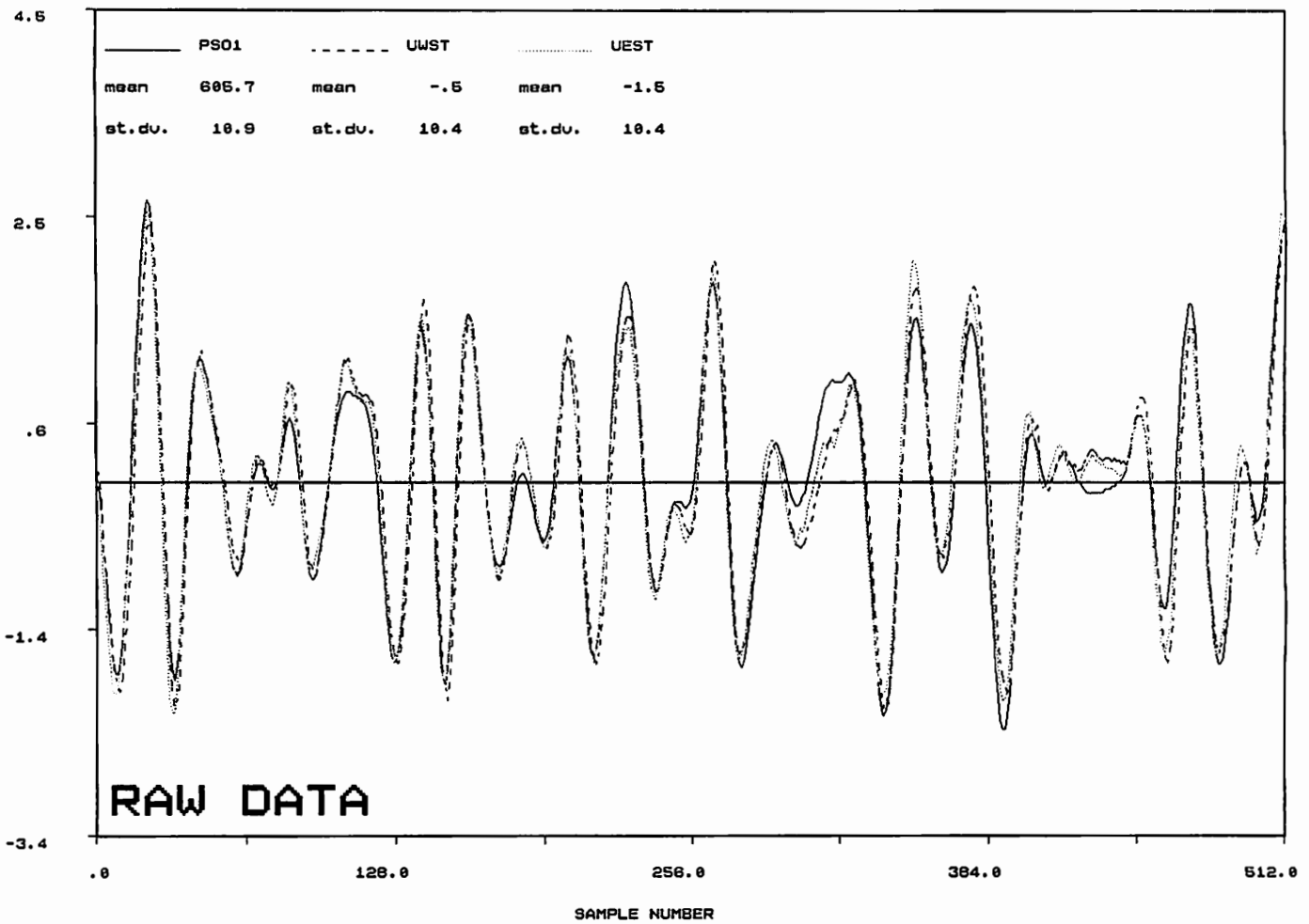


Figure 5. Time series (2 min, 8 sec) of bottom pressure (PS01) and cross-shore velocities (UWST, UEST). Vertical axis units are arbitrary; time axis is in units of data samples (four samples per sec).

surface-wave directional spectra can be estimated, an array of conventional sensors were deployed at the end of Scripps Pier in 6 m of water. Nine capacitor-type, absolute pressure sensors and one open-frame electromagnetic current meter (Clifton and Lowe, 1986) were deployed, with relative spacing shown in Figure 6. Data from this array of sensors were collected using the shelf-and-shore system (Boyd and Lowe, 1985). Data from both DADS and the array were collected for approximately 2 hours on each of 7 days in May and again for several days in August and September. The data from DADS collected in May were processed using incoherent techniques, while those collected in August and September were processed using coherent methods.

The first comparison is between the directly measured pressure and pressure calculated using the measured velocities from DADS and linear theory. Linear theory is known (Guza et al., 1988, and references therein) to accurately relate these variables in similar situations. In terms of the cross-shore, longshore, and vertical velocities (u,v,w) the two cross-shore oriented beams (u_1,u_2) measured

$$u_1 = \frac{(u+w)}{\sqrt{2}} \text{ and}$$

$$u_2 = \frac{(u-w)}{\sqrt{2}},$$

while the longshore beams (v_1,v_2) measured

$$v_1 = \frac{(v+w)}{\sqrt{2}} \text{ and}$$

$$v_2 = \frac{(v-w)}{\sqrt{2}}.$$

According to linear theory, the velocity components u_1,u_2,v_1, and v_2 measured with DADS are related to bottom pressure p by

$$E_{pp}(f) = \frac{(2\pi f)^2}{(gk)^2(1+2\tanh^2 kd)} \left\{ E_{u_1}(f) + E_{u_2}(f) + E_{v_1}(f) + E_{v_2}(f) \right\},$$

where E is the frequency spectrum, f is frequency, k is the wave number given by linear theory, g is the acceleration of gravity, and d is the height of velocity measurements above the bed.

A comparison using incoherent velocity data collected on May 19, 1989 is shown in Figure 7. This data set was selected because the

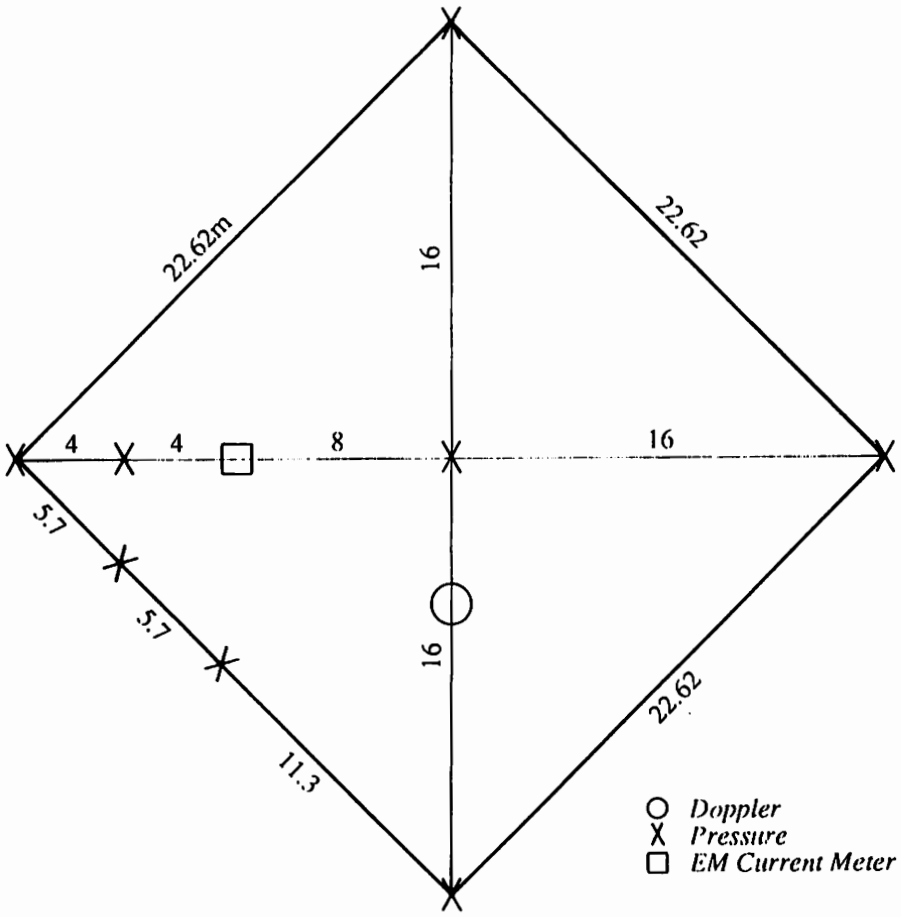


Figure 6. Plan view of instrumentation (pressure sensor array, electromagnetic (EM) current meter and Doppler instrument).

measured noise level above 1 Hz (not shown) is close to that predicted for incoherent processing, suggesting that surface reflections were not a significant problem. The predicted and measured spectra are similar from very low frequencies, about 0.025 Hz (40 s) to about 0.25 Hz (4 s). Over this frequency range, the ratio of the predicted to the measured spectra is about 1.0 ± 0.1. The divergence at the higher frequencies is caused by the noise level (self clutter) inherent in the incoherent signal processing. This noise level is independent of the energy in the signal so one would expect that, as the total energy drops, the frequency range over which there is good agreement will also decrease. We do not yet have sufficient data to verify this expectation.

Comparisons similar to Figure 7 were also made for 3 days when coherent processing was used to obtain the velocities. These 3 days have a wide range (20:1) in the energy density of the spectral peak, including one with an extremely low energy peak of only 2.6×10^2 cm^2 Hz. For the same agreement criterion, the frequency range of coherent processing is 0.1–0.35 Hz, compared to 0.025–0.25 Hz for incoherent processing. As expected, coherent methods appear to extend the high frequency range of agreement because coherent methods do not suffer from the self-clutter of incoherent methods. The poorer performance of coherent processing at low frequencies is probably caused by either surface interference and/or unresolved aliases.

A coherent data set acquired from September 30, 1989, with all discontinuities carefully removed, was also processed. The results (Figure 8) show excellent agreement over the entire frequency band of surface gravity waves. The frequency range of agreement within 10% is about 0.04 to 0.31 Hz. Figures 7 and 8 indicate that both coherent and incoherent systems measure the magnitudes of the wave-induced, oscillating, horizontal components of the nearshore velocity field. No directional information is included in these comparisons.

Low-order moments of the wave directional spectrum were also compared. Only four bottom pressure sensors are required to estimate these moments, and various combinations (sub-arrays) of the nine bottom pressure sensors were used to generate estimates using the methods described by Herbers and Guza (1989). The sub-arrays of pressure sensors yield results in excellent agreement with each other (Figures 9 and 10). These moments were calculated from the DADS data with linear theory. For horizontal separations of the four DADS velocity components that are small compared to the surface wavelength,

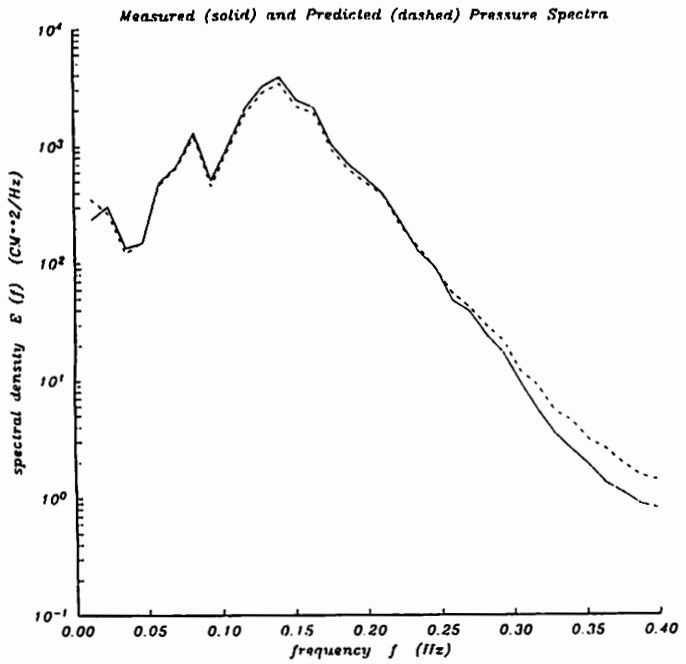
$$\int_0^{2\pi} d\theta S(\theta;f)\cos 2\theta = \frac{C_{uu}(f) - C_{vv}(f)}{C_{uu}(f) + C_{vv}(f)} \text{ and}$$

$$\int_0^{2\pi} d\theta S(\theta;f)\sin 2\pi = \frac{2C_{uv}(f)}{C_{uu}(f) + C_{vv}(f)},$$

where $u = u_1 + u_2$ and $v = v_1 + v_2$, C is the cospectrum, and $S(\theta;f)$ is the normalized directional distribution of wave energy at frequency f. Moments from the array of pressure sensors and DADS are compared in Figures 9 and 10 (incoherent and coherent processing, respectively). Both processing methods yield good agreement over the range of energetic frequencies. The DADS was retrieved and redeployed between May (Figure 9) and September (Figure 10) and was apparently not accurately oriented in September. A 5° rotation in the horizontal plane was necessary for good agreement in September.

Conclusions

DADS has demonstrated the ability to accurately measure low-order directional moments of surface gravity waves in the field. It is not clear whether more detailed information can be obtained from



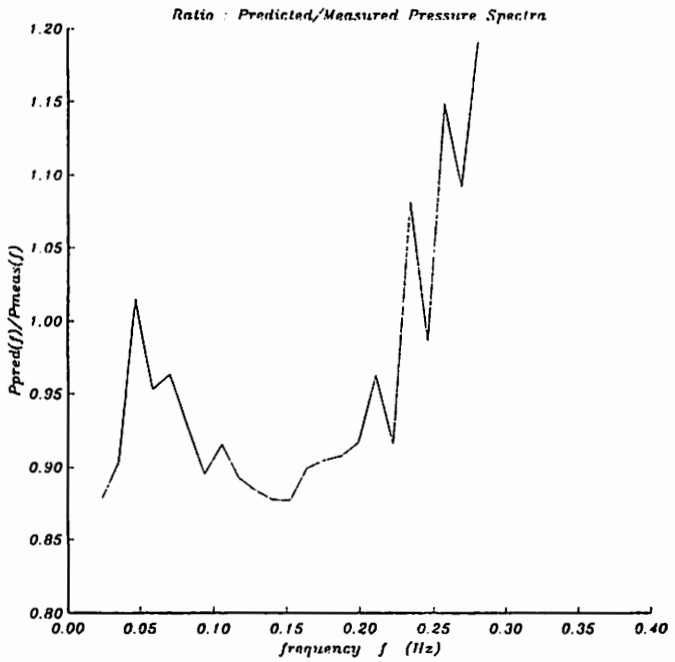
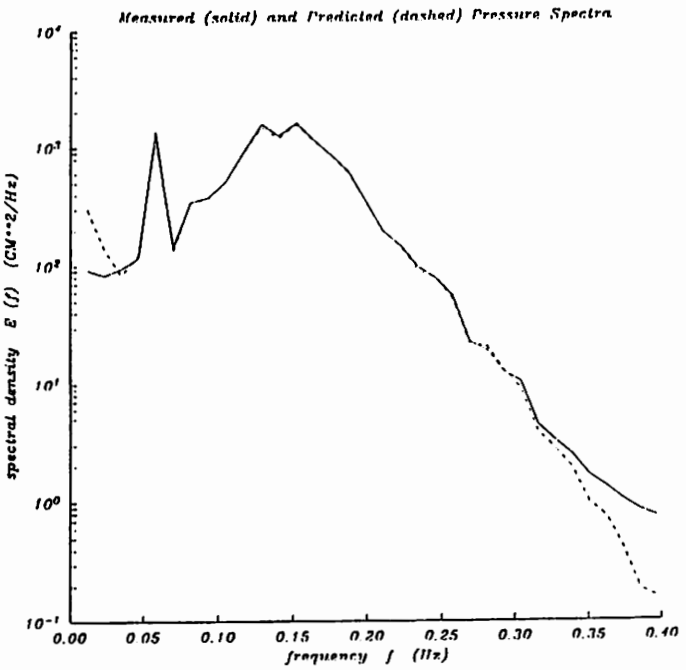


Figure 7. Nondirectional results from May 19, 1989 using incoherent processing. Top: Directly measured (solid line) and calculated from DADS (dashed line) pressure spectra. Bottom: Ratio of calculated/directly measured spectra.



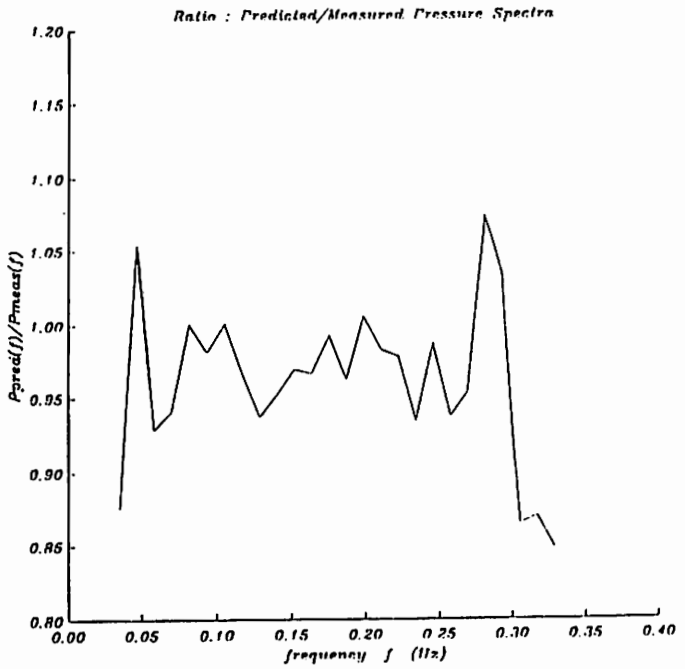


Figure 8. Nondirectional results from September 30, 1989 using coherent processing: same format as Figure 7.

DADS. Two problems have been identified: (1) interference from surface returns and (2) relatively high noise associated with incoherent processing. Both of these problems can be significantly reduced by minor changes in the timing of the system.

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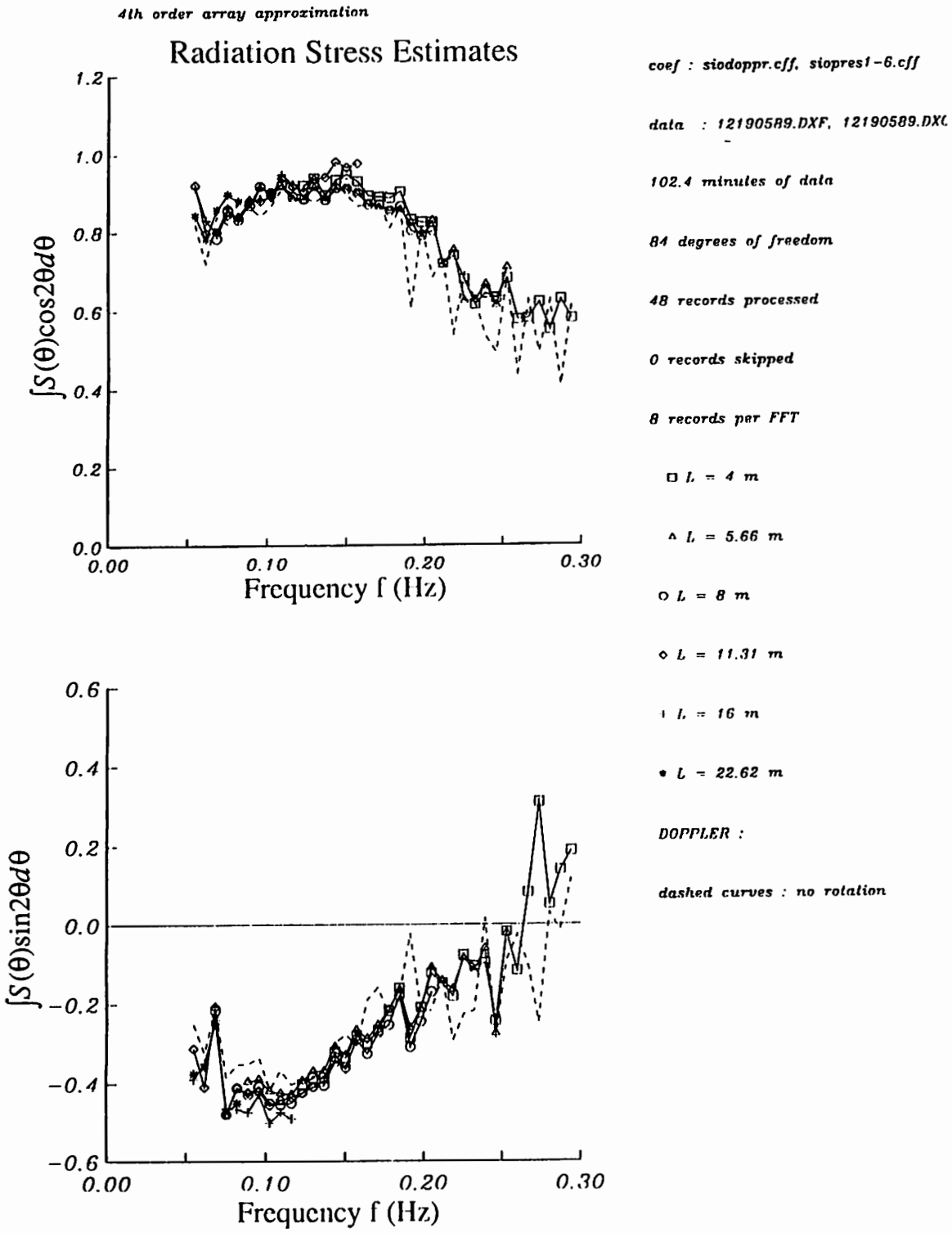

Figure 9. Incoherent technique. Directional moments on May 19, 1989 calculated from pressure sensors (symbols) and DADS (dashed line with no rotation, dotted line is for 5° rotation).

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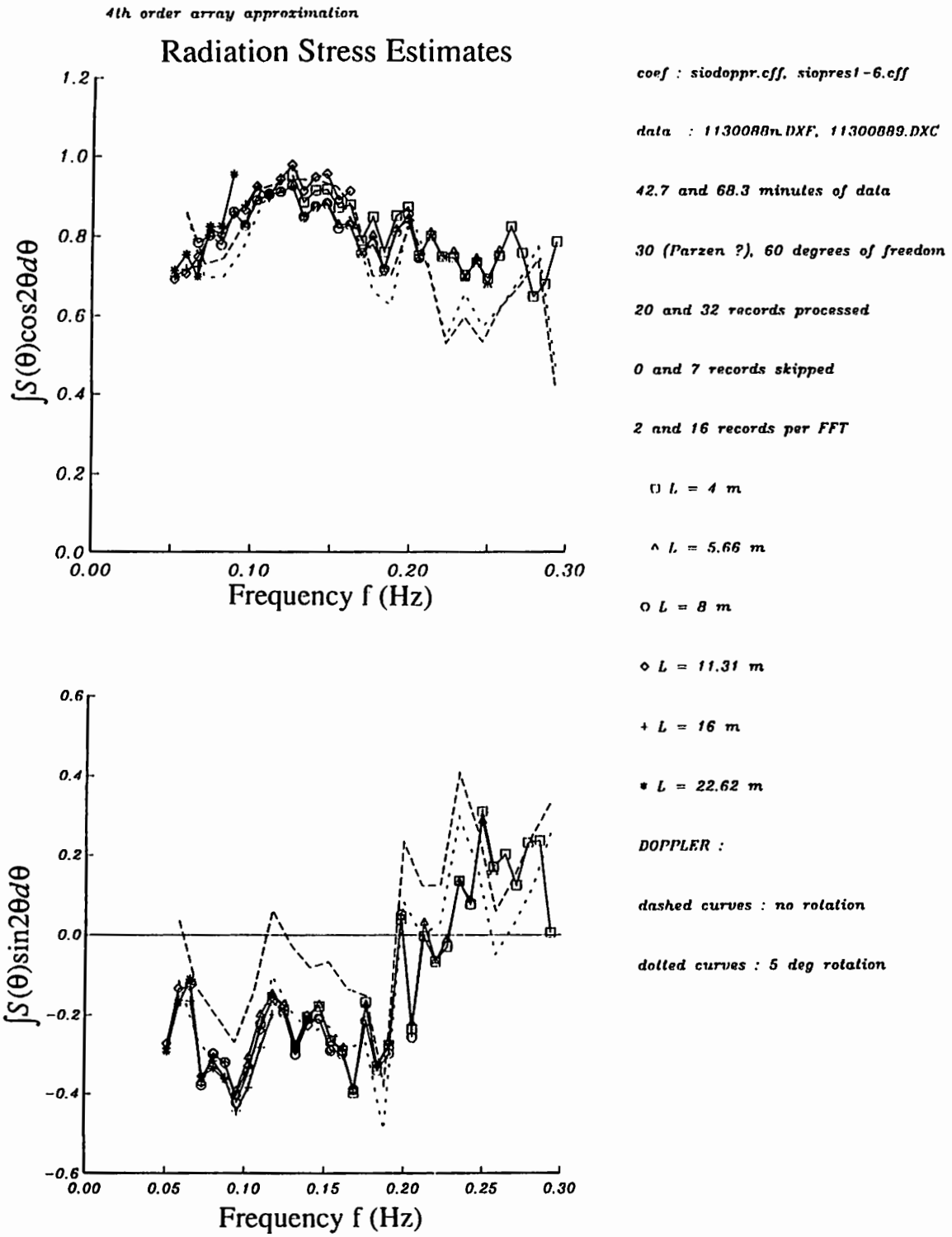

Figure 10. Coherent technique. Directional moments on September 30, 1989; same format as Figure 9.

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System Reliability of Offshore Structures

University of California, Berkeley
R/OE-5
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Alaa E. Mansour and Rabi S. De

System reliability deals with the uncertainties influencing the overall reliability of a structure as opposed to that of individual components. Offshore structures are, in general, highly redundant structures. Failure of an individual member does not imply system failure, and the ultimate capacity of the structure is usually larger than the load at which the first member failure occurs. While redundancy is a much desired "system effect," not all aspects of the multimember situation are beneficial. In particular, in a multimember system, more than one member may initiate failure, resulting in an adverse system effect due to the presence of many members (Ditlevsen and Bjerager, 1986; Cornell, 1987). Because the system effects differ from design to design, structures with the same member-level reliability may manifest widely varying overall structural reliability.

The work carried out in this project can be categorized in three interrelated areas: study of system behavior, wave-load modeling, and application of system reliability-analysis techniques. The reliability of simple, parallel structural systems was studied in order to understand and quantify the system factors influencing the overload capacity and the redundancy of realistic statically indeterminate structures. In contrast to previous studies of ideal parallel systems (e.g., Hohenbichler and Rackwitz, 1983; Guenard, 1984; Stahl and Geyer, 1985; Cornell, 1987; Rackwitz and Gollwitzer, 1988), the present study considered unbalanced parallel systems. In an unbalanced system, the ratio of mean member capacity to mean member force, (i.e., mean safety factor) is different for different members, both in the intact and in the potential damaged states of the structure. For a structure that is perfectly balanced (in the mean), the system effects, including redundancy and overload capacity beyond the first member failure, are strictly due to the randomness in the capacity variables (i.e., they are probabilistic in origin). In contrast, for a more realistic unbalanced structure, the system effects inducing redundancy are both deterministic and probabilistic in origin (De et al., 1989).

In this study, the effects of various parameters were investigated, such as the number of members, postfailure member capacity, and excess design capacity. Simple approximations were developed for estimation of the probability of failure of complex but idealized unbalanced systems. The findings from these near-ideal parallel systems were applied to nonideal redundant systems, in particular, to a fixed offshore jacket structure. Simulation-based, accelerated, system-reliability analysis techniques were investigated. A reduced-space Monte Carlo simulation approach (also known as conditional expectation—see Cornell, 1987; Karamchandani, 1987) was used for reliability analyses for the system-behavior study of idealized structures. This also proved to be a very efficient technique for offshore-structure system-reliability analysis.

On the basis of the preceding investigation of system behavior of idealized "parallel systems," we concluded that the "effective redundancy" of the structure strongly depends on the variability of the load. In general, wave-load variability (due to inherent randomness of the wave-elevation process, statistical uncertainty in estimating sea-state parameters, and uncertainties in prediction of wave forces given a certain sea-state description) was significantly greater than the variability of the capacity parameters, at least in the case of truss-type behavior of offshore jackets. Offshore structures are exposed to several environmental loads acting simultaneously. Loads from waves, currents, and wind are random and tend to be highly correlated because of the common generating and driving mechanisms. Wave loads are often the most dominating environmental load. In the simplest approach to wave-load modeling (henceforth referred to as the "fixed-pattern approach"), the wave load is modeled as a fixed spatial pattern of nodal forces scaled by a random intensity factor (e.g., Guenard, 1984; Nordal et al., 1987). For example, the spatial pattern may correspond to the relative forces produced by the "design wave" at its "worst location" with respect to the structure. The worst location is usually characterized by the position of the wave (during its passage through the structure) where some global load effect (e.g., base shear) is maximum. The random (load) intensity factor for a fixed offshore structure may be base shear—the total lateral load, whose probability distribution can be derived from that of the wave height by using a simple functional relationship between the base shear and the wave height. The variability of the wave-load magnitude (e.g., base shear) depends on the variability of the wave height and the uncertainty in predicting the wave forces given a wave height. The implicit assumption in the "fixed pattern approach" is that the load pattern is independent of the wave height; only the load magnitude is a function of the wave height. Gravity loads are modeled by a deterministic spatial pattern with random (scalar) magnitude. System reliability is estimated under the assumption of "static monotonic" application of the load pattern with random magnitude (i.e., the nodal loads are gradually increased from zero to their final magnitude in a monotonic fashion, always maintaining the same relative nodal-force pattern). The system-reliability framework consists of expressing the system failure event as a combination of component

failure events and using approximate but efficient, reliability computation techniques known as the first-order reliability method (FORM) and the second order reliability method (SORM) (see Madsen et al., 1986; Bjerager, 1989). Several simulation-based, accelerated, system-reliability analysis techniques were also investigated in which the wave load was modeled by a fixed-load pattern scaled by a scalar random variable.

The choice of the wave-load pattern corresponding to the design wave (e.g., the 100-year wave) in the fixed-pattern approach was arbitrary. The load pattern changes significantly with wave height because of the change in fluid kinematics. In particular, waves reaching the deck of the platform produce slamming forces that cannot be modeled realistically by the 100-year-wave pattern. A more rigorous approach, referred to as the "wave-fragility approach," was used to estimate the system failure probability. In this approach, the system failure probability was obtained for each of a set of wave heights. These probabilities were conditional on the wave height, and hence the load variability in each of these system-reliability analyses depended only on the uncertainty in estimating the wave-load magnitude for a given wave height. The conditional system failure probabilities were multiplied by the (discrete) probability levels of the wave heights, and the products were added together to obtain the (marginal) system failure probability. The wave-fragility approach provides insight into the mechanism of failure at each wave height. This approach also provides a basis for calibration of simpler fixed (single) pattern approaches for estimating system reliability.

The wave-load patterns adopted for both the fixed-pattern and the wave-fragility approaches were essentially deterministic. The randomness of the wave load was modeled by a single random variable, namely, the load intensity factor (e.g., the base shear). The nodal wave forces were fully correlated (i.e., if the force is known at one node, the forces at all other nodes are also known). Common functional dependence of the nodal wave forces on the wave height results in a high level of correlation among the nodal wave forces. However, local variations due to the irregular wave elevation, uneven marine growth on the structure, and local uncertainties in wave kinematics and force prediction resulted in less-than-perfect correlation among the nodal wave forces. In response, an analytical form was assumed for the spatial distribution of correlation between nodal wave forces. The parameters of the correlation model included a base (nonergodic level) correlation and rates of decay in each of the three dimensions in space. A framework was developed for the probabilistic description of nodal wave forces, consistent with the prescribed correlation structure and the available information on the base shear distribution. A parametric study was carried out to investigate the effects of variation of the correlation model parameters on the probabilities of both member-level and the system-level failure (any-first-member failure and system failure), including changes in potential failure sequences. The correlation model was calibrated on the basis of the published wave-loading results from the Ocean Test Structure (Dean et al., 1979; Haring et al., 1979; Heideman et al., 1979).

Calibration from limited published data indicated that wave-load representation by a deterministic pattern scaled by a scalar random variable could be satisfactorily used in system-reliability analysis for global truss-type behavior of jacket structures. The investigation clarified issues dealing with spatial averaging involved in the base shear (the chosen global load intensity random variable), and the results suggested at what point the load variability could be reduced for system-reliability calculations.

In view of the importance of the load variability in system-reliability analysis, a new framework for modeling a narrow-band random process is proposed. Unlike the common frequency-domain (i.e., spectral) representation of the random process, the proposed framework is based on a probability-domain (i.e., random-variable characterization) model of the random process. This model is also applicable to non-Gaussian random processes. This is particularly significant, because the wave-elevation process for an extreme sea-state is often non-Gaussian. Moreover, the response of nonlinear systems (e.g., the drag force) becomes non-Gaussian, even if the input wave process is Gaussian. In the proposed model, a random realization of the process is discretized on a wave-by-wave basis, and statistics are collected on the individual wave segments for the duration of the random process. This framework will be applied to model a non-Gaussian wave-elevation process during a storm sea state. The random variables are characterized by their higher-order marginal moments and pair-wise correlation (Winterstein et al., 1989). This representation is suitable for use in either FORM/SORM or Monte Carlo simulation to evaluate the probability distribution function of any response, linear or nonlinear. In particular, base shear probability distribution will be derived. The framework is also suitable for modeling spatial (local) uncertainties in kinematics and force coefficients. By simulation (and, in principle, by FORM/SORM also), spatial correlation between nodal forces can be systematically derived instead of calibrating an assumed analytical model as suggested previously. We expect that—through analysis of similar extreme sea-state data—parameters of this probability-domain model of the short-term sea-state can be functionally related to the long-term sea-state distribution parameters such as periods of significant wave height or average zero-up crossing. At present, this idea is presented as a research tool for better understanding and characterization of load processes. The methodology also demonstrates the potential diverse applications of both the component-reliability and the system-reliability techniques.

For a full report of the research carried out under this project, the reader is referred to De, 1990.

Cooperating Organizations

Stanford University, Department of Civil Engineering, Stanford, California

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Resistance of Offshore Structures to Collision

Jean-Louis Armand

University of California, Santa Barbara
R/OE-6
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Accidental damage to offshore installations can have potentially devastating consequences in terms of human life as well as environmental impact. The evaluation of the response of a structure involved in a collision accident is based on the value of the design load that is used as input in the calculation. The collision energy to be absorbed by the colliding structures is a function of the size and type of the striking vessel, the collision velocity, the nature and extent of contact during collision, and the hydrodynamic forces acting on the ship during contact. Data are available for the size and velocity of the striking vessel and for the nature and extent of the contact. Empirical formulas obtained under simplifying assumptions are presently being used to evaluate the total hydrodynamic forces exerted on the striking ship. Although these results are traditionally used in the design process, they should be limited to small motions and should be carefully validated when large-amplitude motions with sudden velocity changes are involved. Considerable research is still required in this area (Ellinas and Valsgard, 1985). A review of work to date on this topic is presented in Saubestre (1988). A brief outline is given here.

The total mass of the vessel used in the design of collision accidents should include both the mass of the ship and the added mass that accounts for the hydrodynamic forces acting on the ship during collision. The present design rules recommend using the added mass as a constant value that is added to the mass of the ship (40% of the mass of the ship for sideways collision and 10% of the mass of the ship for a forward collision). These values are derived by making very strong linearizing assumptions, and previous work has shown that they are reasonable when the duration of impact is short. In some cases, however, the added mass in sideways collisions may equal the mass of the ship (Ellinas and Valsgard, 1985; Kjeoy, 1982; Motora et al., 1971).

A ship hitting a platform sideways generates the largest hydrodynamic collision load. Therefore, we focused our attention on the problem of determining the sideways added mass.

Motora et al. (1971) used a strip-theory approach, in which the three-dimensional problem is reduced to a two-dimensional problem by assuming that the fluid variations in a direction parallel to the ship are negligible (this is justified for a long ship in sideways motion). The problem was formulated in the frequency domain, and the frequency-dependent added-mass coefficients were determined experimentally. For other shapes, added-mass coefficients can be determined either by the Frank close-fit method or analytically (see, for example, the computation of the sway added mass of a cylinder in Tasai, 1961). Motora et al. (1971) showed that the ratio of external force to the acceleration of the striken ship varies with the time elapsed during collision. On the basis of this finding, these authors introduced an equivalent added mass given by the ratio of the external force divided by the acceleration at the end of collision and showed that the value of the equivalent added mass changes with the duration of collision. If the duration is sufficiently small, the equivalent added mass is equal to the so-called added mass for infinite frequencies, which corresponds to the value of 40% of the mass of the ship. The formulation of this problem is linear, and it uses results that are derived by using linear theory, specifically, the values of the added-mass coefficients at different frequencies. Beck and Liapis (1987) formulated the problem in the time domain. This method is related to treating the problem in the frequency domain by use of Fourier transforms. In the time domain, the motion of the floating body is not limited to a rectilinear motion as it is in the frequency domain. By using the impulse-response function developed by Cummins (1962), Beck and Liapis (1987) obtained numerically the value of the force in the case of a body impulsively started from rest.

The work outlined in Motora et al. (1971) and Beck and Liapis (1987) is very extensive but is limited to a linear approach of the problem of impulsive, transient problems. A simple experiment run in the flume of the Ocean Engineerig Laboratory of a floating cylinder suddenly stopped shows splashing on one side and detachment on the other side. These observations are characteristic of nonlinear effects. A thorough experimental, analytical, and numeric study of the simple problem of a freely floating cylinder in two dimensions can yield a great amoung of information about the added-mass effect. Modeling this effect is crucial for correct assessment of the overall dynamic force exerted on the platform.

A numerical method to characterize nonlinear, transient, free-surface flows, using a mixed Eulerian-Lagrange boundary-integral (MELBI) formulation was originally developed to study steep waves (Longuet-Higgins and Cokelet, 1976). It has since been extended to take into account the presence of submerged or water-piercing bodies and motions that are not periodic in space (e.g., Faltinsen, 1977; Vinje et al., 1982; Lin, 1984; Cointe, 1989a, 1989b). This method represented a breakthrough for the study of phenomena that could not be predicted using linear or second-order theories. Its most important contribution was its capability to similate complex free-surface motion such as breaking. The MELBI

method is particularly well suited for problems in which the only relevant calculations are those concerning the boundaries of fluid domain. We used this method in the present study because the points of interest are (1) the force exerted on the hull of the body and (2) the shape of the free surface.

Our only assumption was that the fluid was irrotational. The problem could therefore be formulated with one unknown, the potential, which verified Laplace's equation. Sources are distributed on the boundary of the fluid domain, and the field equation is transformed by using Green's theorem into an integral equation formulated on the boundary of the fluid domain. This boundary consists of solid boundaries (the side and bottom of the domain and the outline of the solid body) and of a moving boundary (the unknown position of the free surface). The line integral is approximated by using a finite number of distributed sources and linear interpolation of the unknown potential and normal potential between these sources. On the solid boundaries, the normal potential is prescribed. On the free surface, the potential is assumed to be known at the beginning of every time step. The subsequent positions of the sources on the free surface and the values of the potential at these sources are followed in time using a fourth-order Runge-Kutta time-stepping procedure. From the value of the potential and the normal potential on the free surface, the potential on the side of the body is computed, and the force is calculated using Bernouilli's equation.

Although MELBI methods rapidly gained popularity, careful studies were still needed to assess the validity of the computer-simulation results. Special consideration must be given to the intersection points and the extent of the numerical domain (e.g., Lin, 1984; Cointe, 1989b; Dommermuth, 1987). With this in mind, the numerical code MEDUS was created. It solves the fully nonlinear equations of motion within the framework of potential theory in two dimensions. The algorithms used incorporate techniques to account for the simulation of a current, to model the forced- or free-motion of a submerged or water-piercing cylindrical section and to overcome the difficulties associated with the finite extent of the numerical domain and the singular behavior at the body-fluid intersection. Special care was taken to validate each step leading to the analysis of the sideways motion of a cylinder moving sideways. In particular, classical potential, linear and second-order results were recovered (Saubestre 1990 and 1991).

In order to validate computer simulations of the hydrodynamic forces in the case of collision, an experimental device was built to measure the force exerted on a floating cylinder in sideways motion. It consists of a low-friction rail to which a circular or rectangular cylinder is attached. This limits the motion to one degree of freedom. The cylinder can be pulled sideways either by falling weights to measure the effect of a constant force or by a motor to ensure constant velocity. The displacement of the cylinder is measured with position transducers. A portal-type connection links the cylinder to the carriage attached to the rail. Strain gauges forming a Wheatstone Bridge are cemented on the sides of the portal plates. Because of the great amount of stiffness in the vertical direction of the plates, the unbalance in the bridge is directly related to the horizontal (or sideways), transient hydrodynamic force exerted on the body.

A Micro Measurements Group (Raleigh, North Carolina) system consisting of Strain-Gauge Conditioner and Amplifier 2100 was used to modulate the force-measurement signal. This signal and the signal from the position transducers were recorded on a Keithley Data Acquisition 570 system.

To pursue this work, a comparison of the experimental data and of the numerical data should be made to assess the overall validity of these calculations and to estimate the contribution of effects not considered because of the potential-flow assumption. In particular, the contribution of viscous effects should be characterized for different collision velocities.

To take advantage of the facilities of the Ocean Engineering Laboratory at the University of California, Santa Barbara, available to us at no cost, the research was directed toward the study of the hydrodynamic forces exerted on the striking ship. This very important and fundamental problem is addressed in the original proposal.

Cooperating Organizations

The Institut Méditerranéen de Technologie, Marseille, France

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Stability of Seafloor Under Wave Loading—Soil Model Validation and Numerical Solution

University of California, Davis
R/OE-7
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

Chih-Kang Shen

Under wave loading, the safety of offshore gravity platforms and the stability of seafloor slopes is a challenging problem of critical concern to both engineers and environmentalists alike. A reliable and rational method of assessing the impact of wave loading on the above is urgently needed. Development of a versatile soil-behavior model capable of correctly describing the soil response under wave loading and its inclusion in a comprehensive and effective analytical formulation to solve complex boundary-value problems seems to hold the key to a reasonable solution. The current research has taken this broad view with the expectation of developing an integrated approach for dealing with seafloor problems. Tentative conclusions are as follows:

1. The hypoplasticity soil model can be adopted to predict the behavior of silt.
2. Because of the existence of initial shear stress in the sloping seafloor, the seafloor soil in sloping ground is more suseptible to the generation of excess pore-water pressure under wave loading, and thus is more prone to have liquefaction-related failure.
3. The stability of gravity platforms installed in deep water can be analyzed more conveniently by the finite-element computer program (SAC2S).
4. The computer program (SAC2S), which incorporates the essential elements for dealing with complex seafloor soil behavior and loading conditions, can potentially be used as a more rigorous and versatile analytical tool to study wave–soil–structure interaction problems.

It must be pointed out, however, that the results of this research are still preliminary in nature. Additional research on model calibration and prediction under complex loadings and on plasticity solutions for studying gravity platform safety are still underway.

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Waterfront Sheet-Pile Walls Subjected to Earthquake Shaking: Analysis Method

University of California, San Diego
R/OE-8
Project Initiated: October 1, 1988
Project Completed: September 30, 1990

Toyoaki Nogami

Strong earthquake ground shaking in coastal areas has caused severe damage on numerous quay wall structures in the past (Seed and Whitman, 1970). Soils behind quay walls are typically loose hydraulic fills vulnerable to liquefaction in the event of a strong earthquake, and evidence of such liquefaction at or near damaged quay walls has been reported frequently. Therefore, the increase in excess pore-water pressure in the backfill during earthquake ground shaking is a critical factor in the seismic performance of quay walls. Currently, however, the mechanism that leads to damage of quay walls during earthquake ground shaking accompanied by a build-up in the pore-water pressure is not well understood. Furthermore, a rational method of analysis that accounts for all the key factors, including an increase in excess pore-water pressure, is not available to evaluate how a quay wall and backfill system behaves during an earthquake. As a result, no satisfactory rationale has been developed for designing waterfront earth-retaining structures that resist earthquakes. The objective of the research reported here was to develop a rational analysis tool that could be used to study the behavior of sheet-pile walls during earthquake ground shaking. Details of the development of the formulation are described in a comprehensive report separately submitted to the Sea Grant Program.

Major factors affecting the performance of waterfront sheet-pile walls are nonlinear soil behavior, hydrodynamic force, development and dissipation of excess pore-water pressure in soil mass, and nonlinear soil-wall interaction. The finite element method can rationally accommodate those factors in the analysis and therefore was developed for analyzing the behavior of sheet-pile walls subjected to earthquake shaking. Submerged soil was treated as a fluid-saturated two-phase porous medium. The so-called U-u formulation (Zienkeiwicz and Shiomi, 1984) based on Biot's formulation of the dynamic response behavior of a fluid-saturated mixture (Biot, 1956) was used for the finite element formulation. Nonlinear behavior was implemented in the soil skeleton stress-strain relationship and the soil-wall interface stress-strain relationship. The seawater at the front side of the wall was assumed to be incompressible and was idealized as added masses

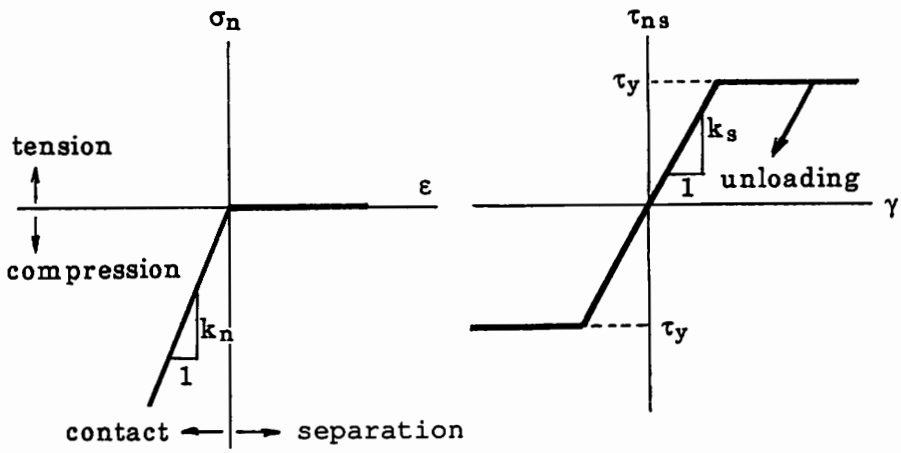

Figure 1. Stress-strain relationship at soil-wall interface.

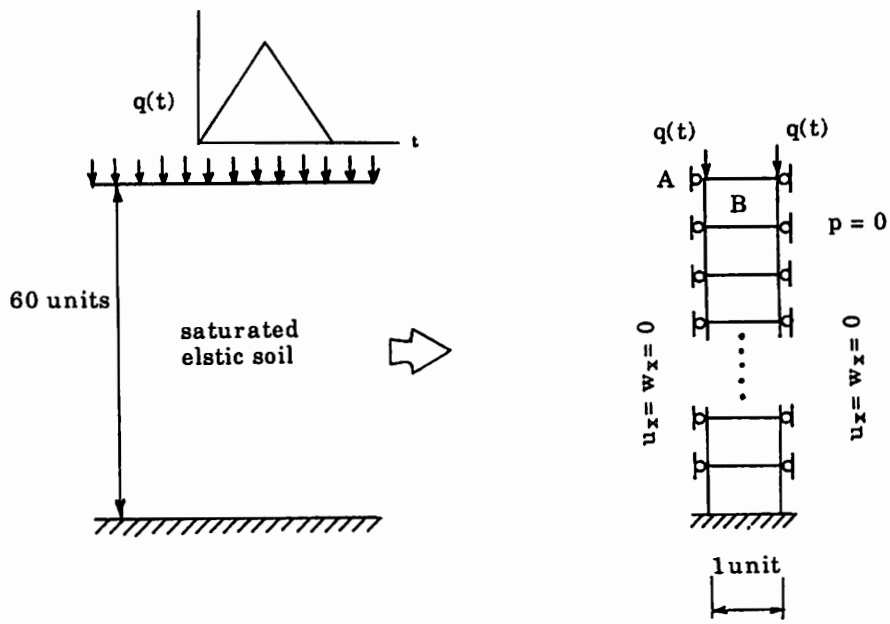

Figure 2. Conditions and finite element model used for verification study.

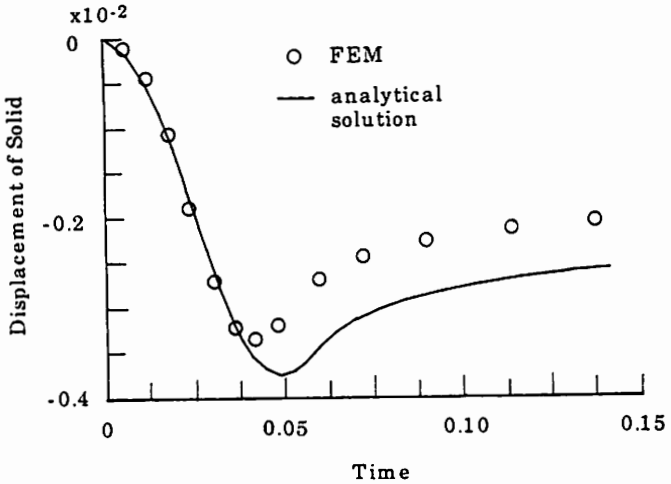

Figure 3. Variation of displacement of solid at surface with time.

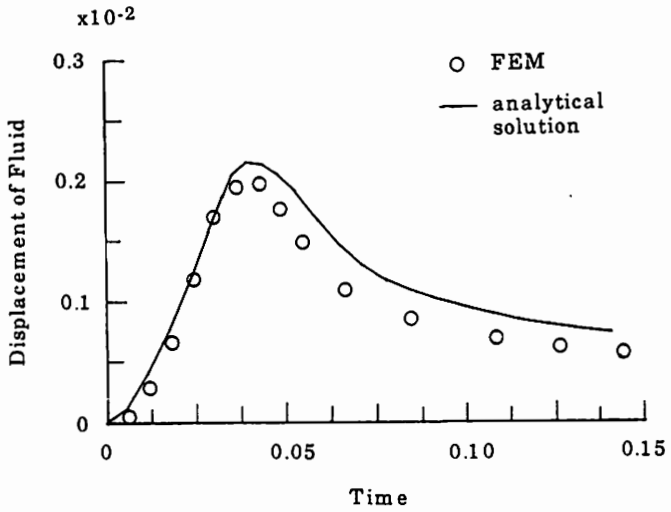

Figure 4. Variation of displacement of fluid at surface with time.

attached at the nodes along the water-wall and water-soil interfaces. Special consideration was given to the treatment of the outer edges of the finite element region to take into account the infinite lateral extent of the two-phase mixture soil domain. Nonlinear analysis of the incremental quantities was performed in the time domain by using Wilson's θ method.

In the present formulation, rather simple elasto-plastic stress-strain relationships were considered for the soil skeleton; more complex and sophisticated ones will be considered later. Thus, the Mohr-Coulomb yield criterion, and modified Von Mises and modified Tresca yield criteria were adopted. When these criteria are used, the yield surface in the s-space is cone shaped, so that the size of the yield surface depends on the confining pressure. It was assumed that plastic strains were induced according to the flow rule together with the associate law and kinematic hardening. Effective stresses acting on the soil-wall interface were decomposed into the effective normal stress (σ'_n) and shear stress (τ_{ns}). Stress-strain relationships for those stress components ($\sigma'_n \approx \varepsilon$ and $\tau_{ns} \approx \gamma$) were assumed to be as shown in Figure 1. No coupling between those two behaviors was considered. Joint elements to reproduce such behavior were formulated in the finite element scheme and placed along the soil-wall interface.

Analytical solutions developed for the fluid-saturated porous medium are available for very simple cases. Formulations and a computer program developed for this study were verified by comparing the values computed by using the finite element program developed and available analytical solutions. One of the cases considered for verification studies was a triangular pulse applied at the ground surface as shown in Figure 2. The analytical solution for this problem was obtained by Simon et al. (1984). Figures 3–6 show the computed results. Agreement between the two computed results was reasonably good.

The magnitude 7.7 Nihonkai Chubu Earthquake hit the Akita Port, which was about 100 km from the epicenter. As a result, a sheet-pile wall of Oohama No. 2 Wharf was displaced permanently toward the sea side by 1.1–1.8 m and was cracked because of excessive displacement (Figure 7). Sand boils and settlements observed at the apron indicated that liquefaction was induced in the backfill during the earthquake shaking. The behavior of Oohama No. 2 Wharf during the earthquake shaking was analyzed by

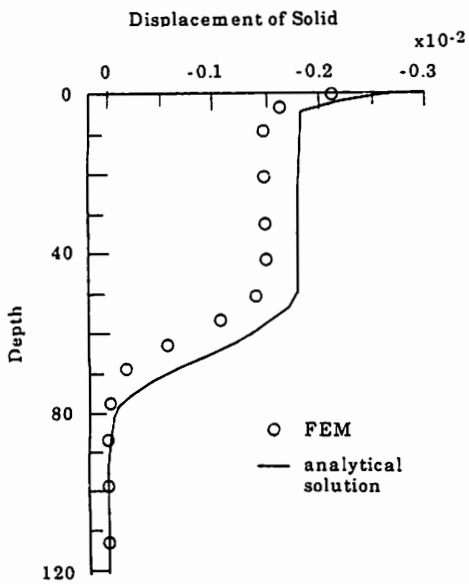

Figure 5. Variation of displacement of solid with depth at t = 0.12.

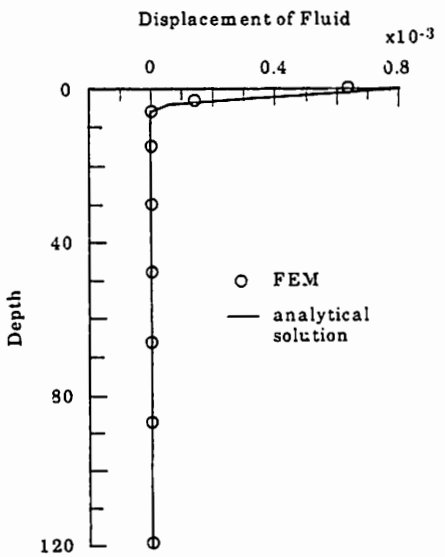

Figure 6. Variation of displacement of fluid with depth at t = 0.12.

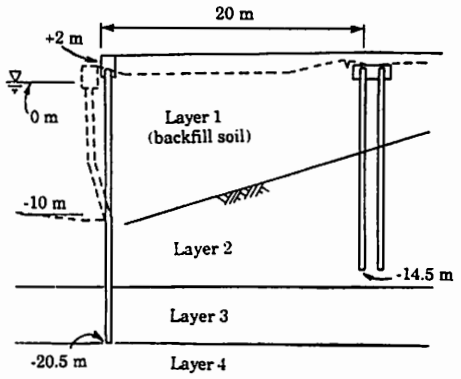

Figure 7. Anchored bulkhead of Oohama No. 2 Wharf.

using the finite element program we developed and data provided by the Port and Harbor Research Institute. Figure 8 shows computed time histories of excess pore-water pressure at locations D and F indicated in Figure 9. As is seen, the backfill is liquefied about 2.4 sec after the earthquake shaking starts. Large permanent displacement develops in the backfill soil because of liquefaction, and thus the wall is displaced excessively as shown in Figure 9. Such a large permanent displacement results in the maximum bending stress at the end of the shaking, 49,000 kpa as shown in Figure 10, exceeding the yield bending moment of the wall (= 30,000 kpa).

Cooperating Organizations
Kajima Corporation, Japan
Port and Harbor Research Institute, Japan Ministry of Transport

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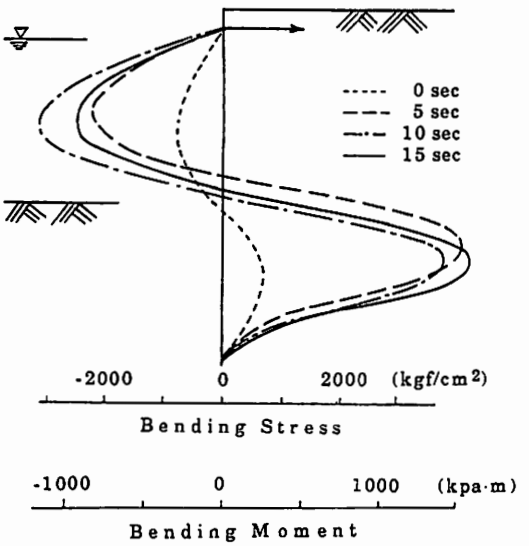

Figure 10. Bending moment and stress induced in sheet pile.

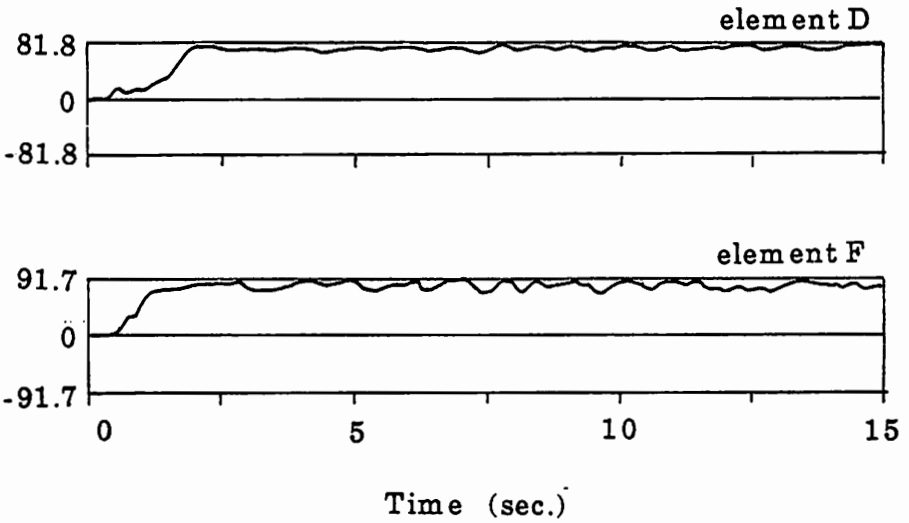

Figure 8. Time histories of excess pore pressure behind the wall.

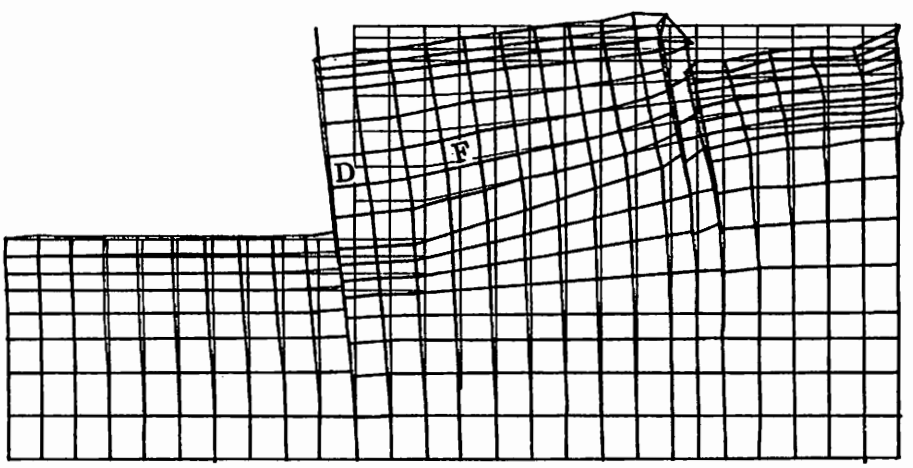

Figure 9. Computed displacements at 15 seconds of earthquake shaking.

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"Black Smoker" Vents for Ocean Thermal Power

Universiity of California, San Diego
R/OE-10
Project Initiated: October 1, 1986
Project Completed: December 31, 1990

Victor C. Anderson

Introduction

Deep seafloor "black smoker" vents emitting very hot water at high flow rates offer concentrated sources of hundreds of megawatts of thermal power. The preliminary engineering analysis of a seafloor power plant indicates that competitive power costs could be achieved even when the cost of several hundreds of miles of submarine power cable is included. The hypothesis addressed by this proposal was that temporal characteristics of the "black smoker" vents are amenable to their exploitation for seafloor geothermal power conversion plants. The approach to be used was development of an autonomous instrument incorporating a scanning short-range pulsed Doppler sonar and a mechanically scanned temperature sensor to periodically profile the thermal flow associated with a "black smoker" vent over periods of up to one year. It was hoped that the instrument would be deployed in cooperation with ALVIN dives or Deep Tow investigations in hot vent areas under other funding. The resulting data would provide essential engineering data relating to the feasibility of seafloor geothermal power conversion plants.

With support from California Sea Grant, I and graduate students Sean Wiggins and William Comeau have created a device to measure the long-term pattern of hot water discharge from a black smoker vent. Named VEMON, for vent monitor, the device is designed to sit next to a black smoker and measure temperature and flow rates at intervals for a year. The objective is to calculate the heat flux (heat output per unit area per unit time) and observe how it changes with time.

VEMON uses a device called a Doppler sonar transducer to measure velocity without disturbing the flow. The device emits a sound pulse of a known frequency and detects the multiple echoes reflected back from particles in the plume. Using Doppler physics, the change in frequency of the echoes reveals the speed of the fluid in which the particles travel (Ware, 1992).

The thermocouple array is oriented to sweep horizontally through the plume after the velocity measurements are made. A temperature profile could be taken every four hours with a single sweep of the arm, stopping at 1.8° increments. A spherical pressure vessel houses the computer control and data storage elements.

The VEMON project awaits further testing and deployment. When it is put in place, it will be the first step towards showing the commercial viability of hydrothermal vents.

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Methodology for Assessment by Regulatory Bodies of the Safety of Existing Steel Offshore Platforms

University of California, Berkeley
R/OE-11
Project Initiated: October 1, 1988
Project Completed: March 31, 1991

Robert G. Bea and Ben C. Gerwick

A steel jacket platform is typically constructed of three main parts: deck structure (air-above water), jacket structure (water medium), and foundation piles (soil medium), as shown in Figure 1. The jacket structure consists of a three-dimensional, tubular space frame, supported vertically and laterally by steel tubular piles driven through jacket legs into foundation soils. The deck structure supports the production, drilling, living, transportation, safety, and other facilities.

Such platforms have been in use for more than 45 years in the Gulf of Mexico and for a lesser period in other offshore areas around the world. The structural configurations of the platforms vary significantly. Variations in their design criteria are the results of differences in water depth, environmental and geotechnical parameters, construction and installation equipment selected, fabrication technology of the period, operational criteria, and the difference in design philosophy (American Petroleum Institute, 1989a, 1989b).

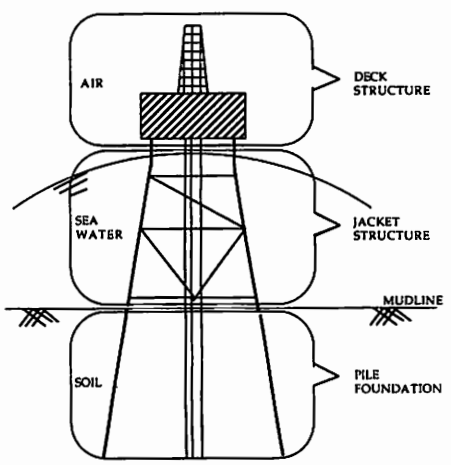


Figure 1. Major parts of a steel jacket platform.

The United States has more than 4400 offshore platforms (Table 1). Many of these platforms (37%) have been operating beyond their usual design life of 20 years (Dyhrkopp, 1990), and some have suffered damages due to corrosion, fatigue, dropped objects, and collision with boats. Thus, they do not necessarily meet the acceptability criteria (safety standards) existing today.

The various regulatory bodies (U.S. Geological Survey, 1979; Minerals Management Service, 1988) and operators are responsible for the safety of these platforms in continued operations, which includes safety of life, safety against pollution, and safety against loss of resources and property. Thus, a well-defined methodology is needed to provide consistency in periodic assessment of the safety of a large number of existing platforms.

The prime objective of this project was to establish a practicable methodology for the safety assessment of existing platforms. The other objectives were to develop a simplified technique for evaluation of reserve strength ratio (RSR), an index of the structural capacity of a

Table 1. Platforms Operating in the Gulf of Mexico-Outer Continental Shelf

S. No.	Age Group (yr)	Year Installed	Major #1 Platforms	Minor #2 Platforms	Total Platforms
1	>25	Before 1965	370	646	1016 (23.1%)
2	20–25	1965–1970	298	329	627 (14.3%)
3	15–19	1971–1975	290	178	468 (10.6%)
4	10–14	1976–1980	418	301	719 (16.3%)
5	1–10	After 1980			1570 (35.7%)
Total					4400

Note. Based on statistics given in Dyhrkopp 1990.
#1 = Platforms with six conductor slots or more, or with at least two pieces of production equipment.
#2 = Smaller platforms, quarters platforms, or equipment platforms. In addition, approximately 148 platforms are installed in the Gulf of Mexico each year.

Table 2. Possible Failure Modes and Mechanisms

Bay Type	Type of Mode or Mechanism	Storm Wave Direction
Deck Bay	Yielding of all deck legs	Diagonal
	Failure of deck leg-pile connection	Diagonal
Jacket Bay(s)	Buckling or yielding of all diagonal braces and jacket legs in the vertical frames between two adjacent horizontal levels	Orthogonal
	Joint failure in addition to buckling and/or yielding of one or more braces	Orthogonal
Foundation Bay	Yielding of all piles	Diagonal
	Pullout / plunging failure of piles	Diagonal

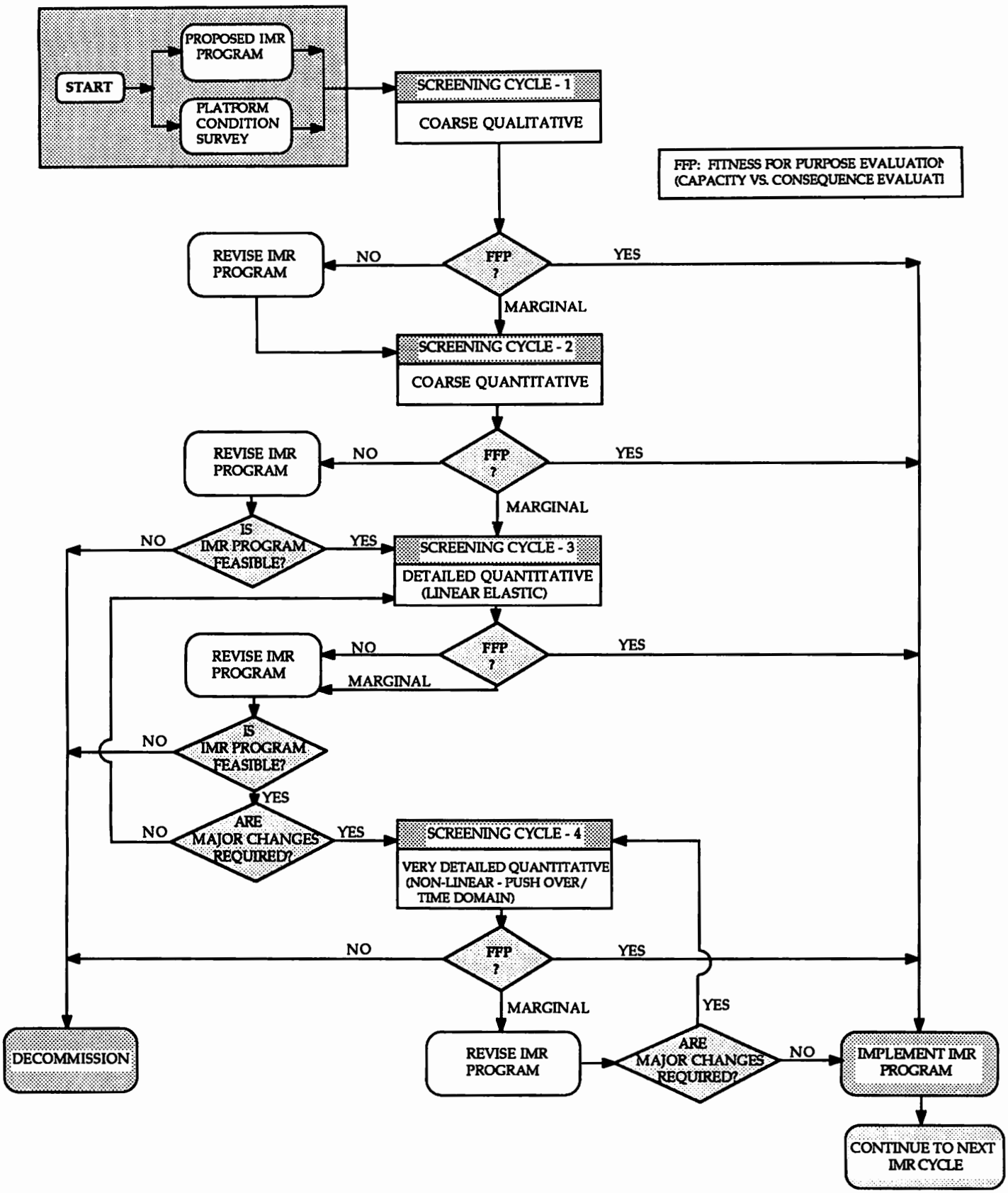


Figure 2. Algorithm for safety assessment of offshore platforms. IMR = Inspection, Maintenance, Repair; FFP = Fit for Purpose.

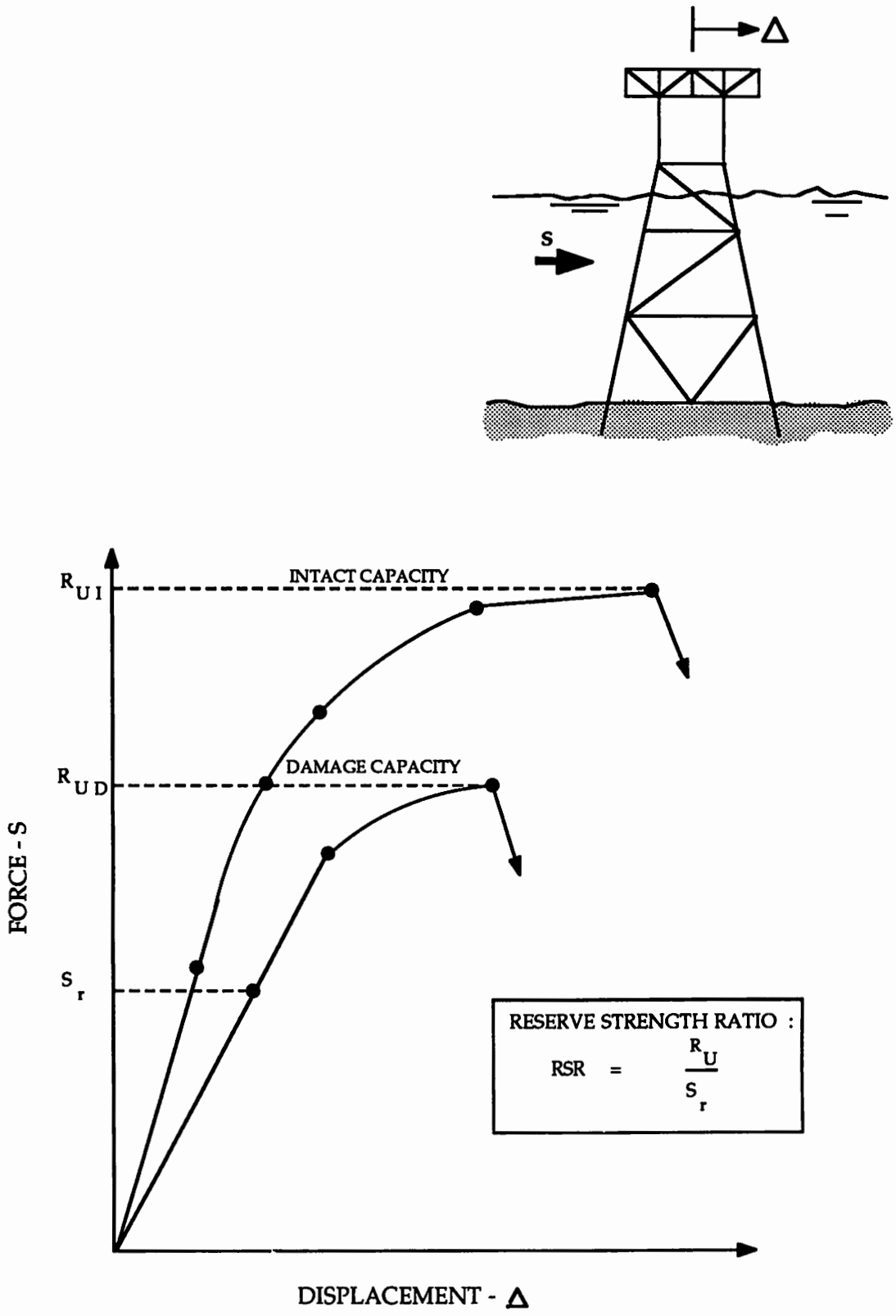


Figure 3. Definition of reserve strength ratio (RSR). R_u = ultimate capacity, and S_r = minimum reference force.

platform; and to develop a basic formulation of a computerized knowledge-based expert system for organizing knowledge components of the methodology.

In order to be classified as suitable for service, a platform should be structurally sound and should not pose undue hazards to the personnel, environment, and property. A four-cycle safety assessment methodology has been developed (Figure 2). This methodology is based on a comprehensive evaluation of the suitability for service of the candidate platforms at one or more of the four cycles. On the basis of such an evaluation, the platforms are categorized as "fit for purpose" (FFP), "marginal," and "unfit for purpose" (UFP).

In evaluations of the platform's suitability for service, the three most important evaluation criteria are: the loadings imposed and induced in the structure; strength and capacity characteristics of the structure; and the potential consequences if the structure fails to perform satisfactorily. The loading on the structure and the strength of the platform are combined and expressed through the reserve strength ratio (RSR = ultimate capacity/minimum reference force), which represents the overload capacity of the platform (Figure 3). The consequence level of a platform essentially depends on the functions of the platform, the operational philosophy followed, and the importance of the platform to functioning of other platforms in the vicinity. An overall decision on the suitability for service of a platform at a level is then based on a comprehensive assessment of its capacity (expressed by RSR) and consequences. An evaluation of feasibility of an IMR (Inspection, Maintenance, Repair) program to maintain the safety level or to upgrade the safety of a platform is then done.

The first step in the safety assessment process (screening cycle 1) is to screen the platforms according to the need for an in-depth investigation of their suitability for service in continued use. This step is an attempt to approach such a decision by using the same factors that would be evaluated by an experienced offshore engineer, yet the process is organized so that it can be applied by a relatively inexperienced yet competent engineer in a methodical manner.

In phase A of screening cycle 1, the emphasis is on the major factors that individually may significantly affect the structural integrity and safety of a platform, and may require further investigation. The major criteria that influence load and strength levels of a platform are evaluated in a logical fashion (Figures 4 and 5). If by a single criterion, a significant increase in load level or a significant decrease in strength level of a platform is likely, the platform exits the process and is further evaluated at cycle 2. In phase B of cycle 1, a matrix procedure is used to identify those platforms with questionable structural integrity and safety due to a cumulative effect of various factors that require further investigation. This phase is based on a qualitative assessment of the capacity and consequence levels. Then, on the basis of these two parameters, a subjective judgment of the safety of a platform is made (Figure 6). The comprehensive grades for capacity and consequence levels obtained from matrix evaluation are subjectively categorized as very low, low, medium, high, or very high. Then these qualitative levels are compared in a capacity-consequence diagram (Figure 7) to make a decision on the overall safety of a platform.

Table 3. Failure of Platforms in the Gulf of Mexico due to Hurricanes (1947–1990)

Platform Type	No. of Platforms	Water Depth (ft)	Failure Mode
Single-well caisson	2	102	Brace-caisson connection
Tripod-well protector	6	30–125	Pile yielding
Four-leg well protector	6	60–92	Pile pullout, braces pullout (100-yr), joint failure, pile yielding, mudslide (1–100 yr, 60 ft water depth)
Four-leg tender	4	50–192	Pile yielding
Four-leg header	1	30	Corrosion and cracks in braces
Six-leg tender	2	60	
Six-leg self-contained	1	87	
Eight-leg tender	7	50–215	Previously damaged, braces failure, deck leg shear, joint failure, vertical collapse
Eight-leg self-contained	5	172–327	Braces failure, pile yielding, mudslide (3 no. 280–327 ft in 100 yr)
Eight-leg central facility	3	51–95	Storm and barge simultaneous, corrosion, holes in braces, broken/missing members
Ten-leg self-contained	1	87	
Total	38	30–327	

Screening cycle 2 of the process is based on application of simplified (coarse) quantitative evaluation to determine if the platform has sufficient reserve structural and foundation capacity, when compared with the reference level forces. The emphasis is on possible failure modes and mechanisms in the three parts of a platform as shown in Table 2, which is based on a detailed review of past failure of platforms in the Gulf of Mexico (Table 3). The coarse quantitative evaluation of capacity is aimed at determination of its "lower bound" estimate. The lower bound estimate is based on component strength of members and ignores the "system effect" (i.e., the structural redundancy in the platform). The simplified evaluation is based on comparison of load and strength patterns for a platform, which are developed by determination of load and ultimate limit strength levels for each bay of the platform (Figure 8).

At cycle 2, the consequence level of a platform is evaluated by using utility theory-decision analysis (Keeney and Raiffa, 1976; Bea et al., 1984; Ang and Tang, 1984). The potential consequences are assessed in monetary terms and converted to utility scale as shown in Figure 9. The fitness-for-purpose evaluation at this cycle is based on a quantitative characterization, which includes uncertainties in loading, capacity, and consequences; safety index; and expected maximum force and reference level force on a platform. An example representation of this characterization is shown in Figure 10 (Bea, 1990). It permits the establishment of acceptable, marginal, and unacceptable combinations of RSR and consequences for given force ratios and loading-capacity-consequences uncertainties.

Screening cycle 3 is applied on a reduced number of platforms and is based on essentially the evaluation process that would be used by a verification agent for a new platform. The capacity level at this cycle is evaluated by using conventional linear structural analysis. After evaluation of RSR, a decision on overall safety of a platform is made on the basis of a capacity-consequence diagram similar to that shown in Figure 10. Screening cycle 4 is the application of a system analysis, based on a nonlinear analysis, that looks at the redundancy level of a structure and the postultimate capacities of members to evaluate the possibility of progressive collapse of the structure. At this stage, the degree of damage tolerance of the system is emphasized. At the simplest, the analysis can be done by performing member-replacement "static push-over analysis," by monotonically increasing the lateral load on the platform and determining member response (Moses and Stahl, 1978; Lloyd and Clawson, 1983; Moan et al., 1985; Bea et al., 1988; Stewart et al., 1988). The static push-over RSR does not give accurate results because the transient, dynamic, and cyclic nature of wave loads and the potential degradation in capacity of elements during intense cyclic loading are neglected. These deficiencies can be addressed by using time-history nonlinear analyses. However, such analyses are extremely difficult and time-consuming and thus are used only for selected platforms.

By such a four-cycle process, a detailed evaluation of reserve strength and consequence levels for the platforms is required only for a few platforms. When a platform is classified as marginal or UFP, at each of the four cycles, the next step in the process is to make a decision on the feasibility of the IMR program proposed for the candidate platform. The IMR program plays a key role in maintaining the safety of a plafform in

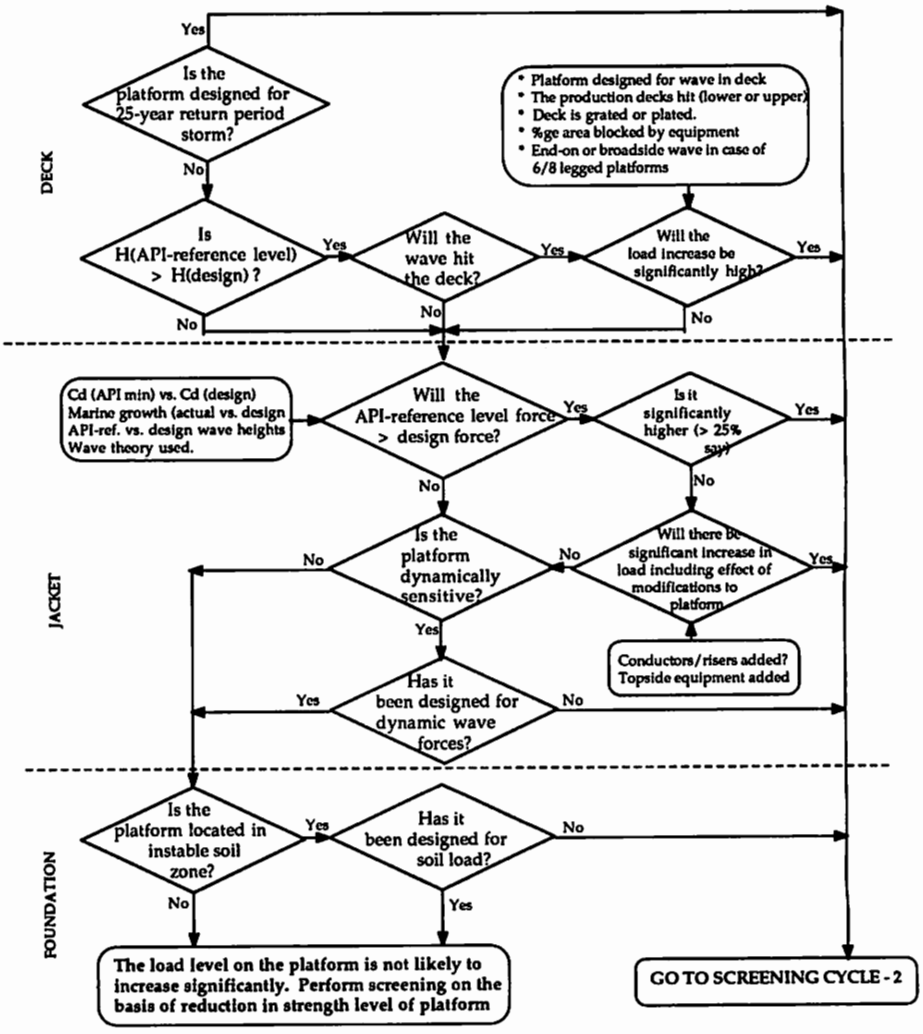


Figure 4. Screening cycle 1A based on potential for significant increase in load level.

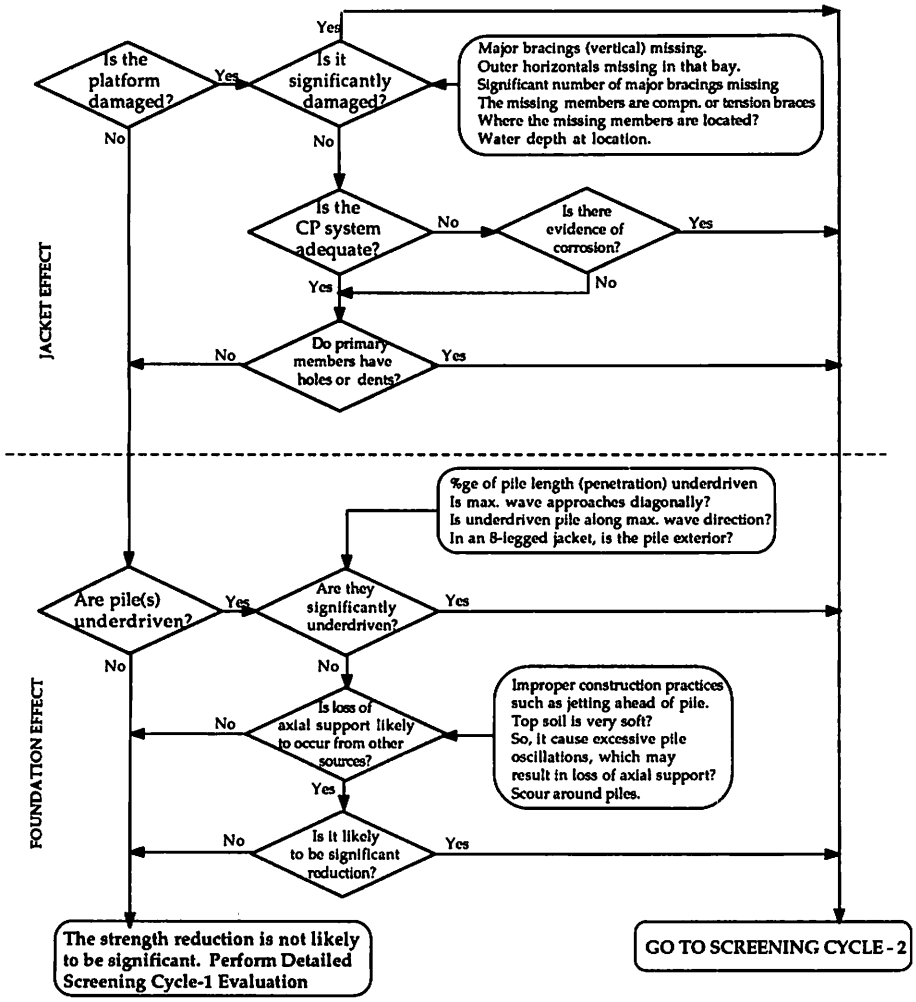


Figure 5. Screening cycle 1A based on potential for significant decrease in strength level.

continued operations. A platform classified as marginal or UFP at any level will need revision of its IMR program and evaluation of the program's feasibility. If the revised IMR program is found to be adequate, then the fitness of the platform in the upgraded condition would be evaluated at the same level or at the next screening level. If the revised program is insufficient, then the platform may be considered for decommissioning to avoid the negative consequences.

The alternative ways of upgrading of a platform to bring it to acceptable safety level are shown in Figure 11. The best solution depends on the characteristics of the particular platform considered. The other alternative is upgrading of the acceptability criteria because of the availability of improved data for a platform, which results in reduction in uncertainties in load, capacity, and consequences. This may result in repositioning of band, as shown in Figure 12. Thus, management of consequences and uncertainties in load and capacity are likely to have a significant effect on the acceptability criteria for safety of a platform.

An effort has been initiated in this project to develop a basis for preparation of a knowledge-based expert system for this methodology. The use of an expert system shell has been identified and initiated. The major components of such a system and their integration with the other software packages are shown in Figure 13 (Aggarwal et al., 1990). The major effort lies in development of knowledge base, user interface, and interfaces with other software and database packages.

It is expected that the end product of this project will be a useful tool for enhancing the understanding of structural system safety and will help the regulators and operators in making a decision on the safety of offshore platforms.

Cooperating Organizations
Belmar Engineering, Inc.
California State Lands Commission
Chevron Oil Company
Griff C. Lee, Inc.
Marathon Oil Company, Houston

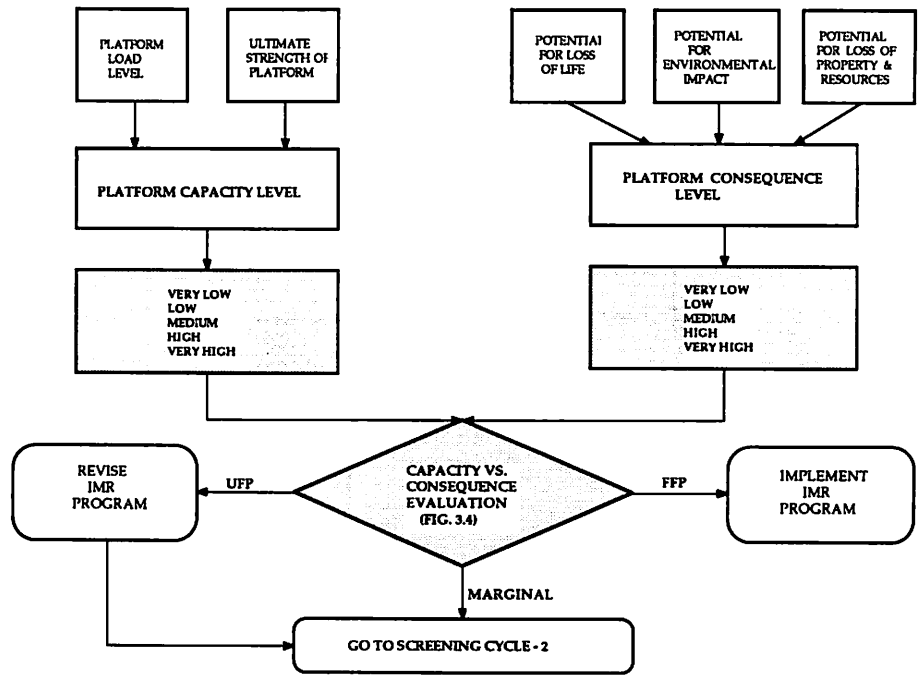


Figure 6. Algorithm for safety assessment of a platform at screening cycle 1B.

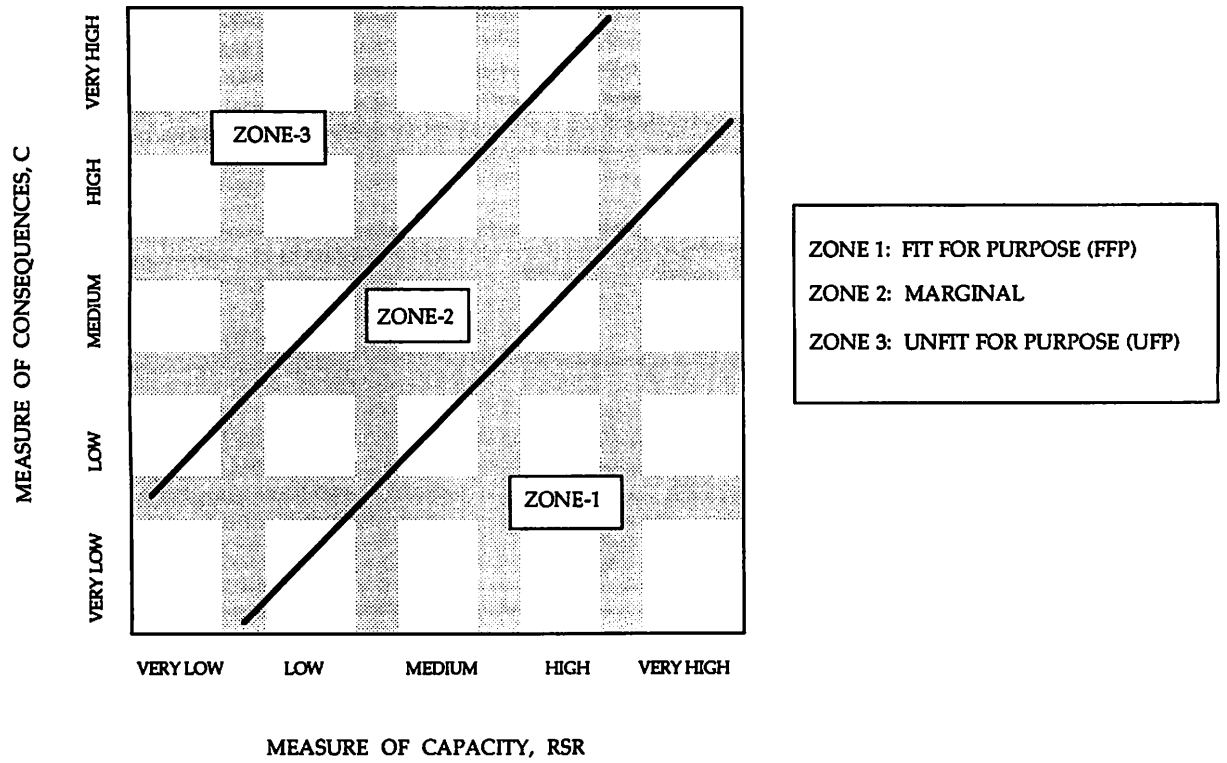


Figure 7. Capacity-consequence evaluation (screening cycle 1B).

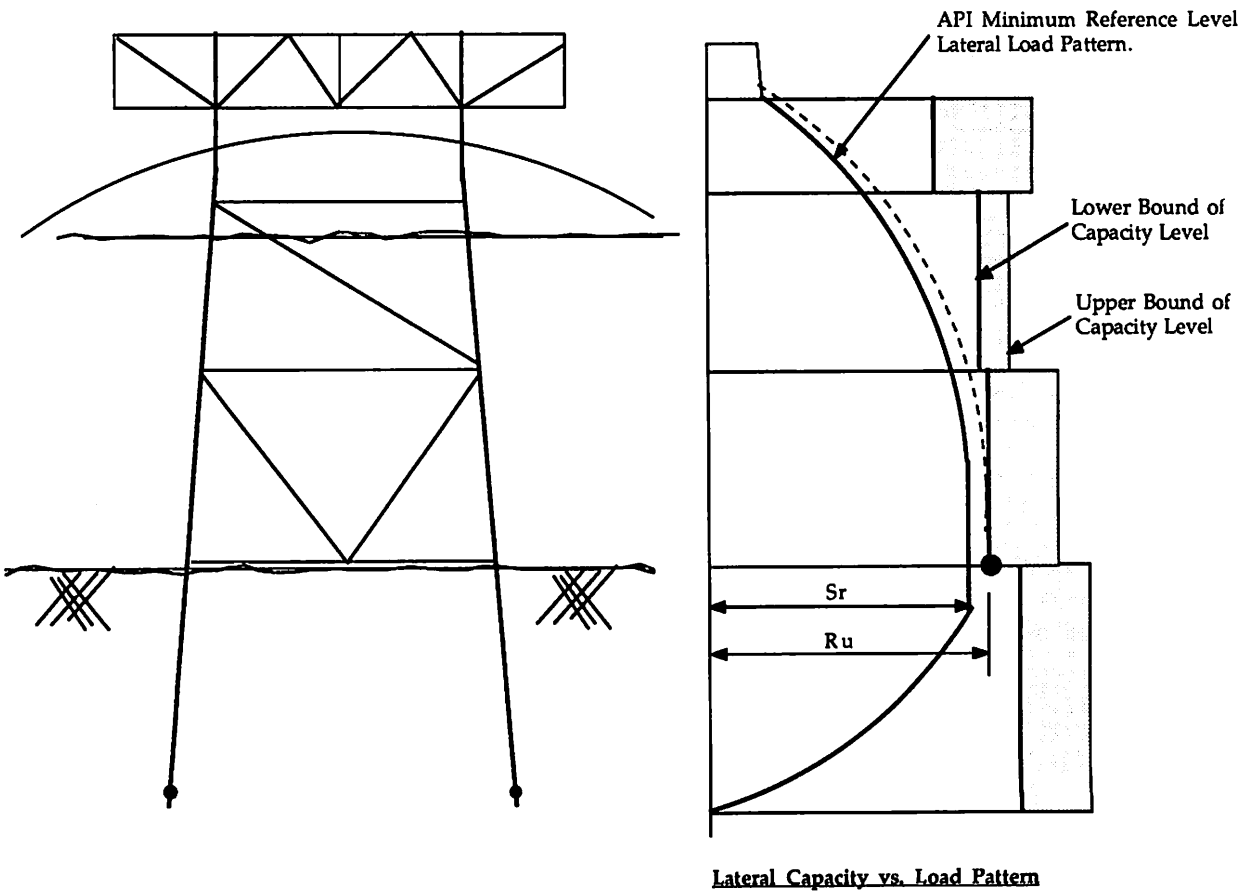
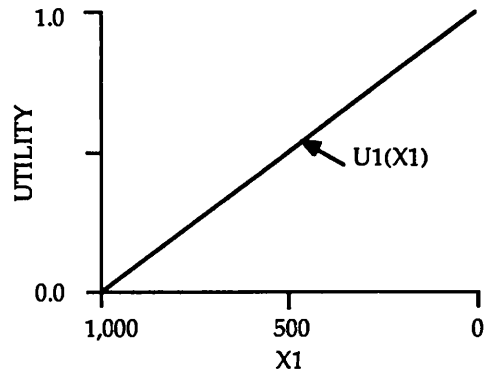


Figure 8. Coarse quantitative evaluation of RSR at screening cycle 2.

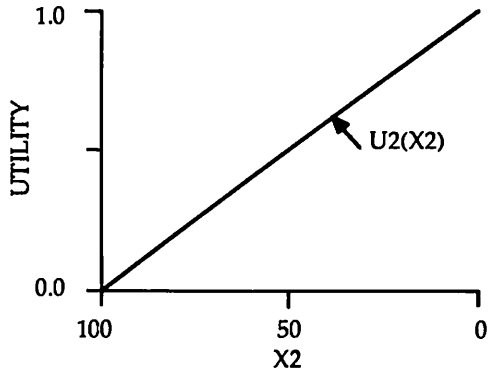
A) COSTS (MILIONS $), X1

UI (XI) = 1.0 - X1/ 1,000



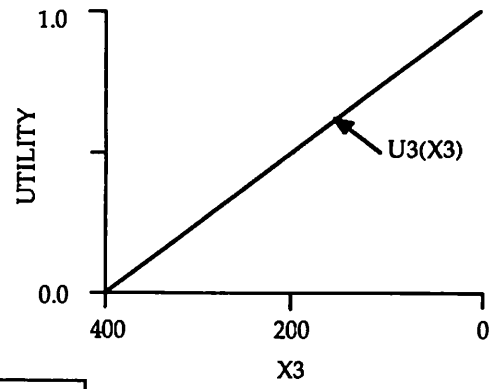
B) INJURIES (NUMBER), X2

U2 (X2) = 1.0 - X2/ 100



C) ENVIRONMENTAL IMPACT (100 BARRELS SPILLED), X3

U3 (X3) = 1.0 - X3/ 400



$$U = \frac{K1}{\Sigma K} U1 + \frac{K2}{\Sigma K} U2 + \frac{K3}{\Sigma K} U3$$

Figure 9. Utility-based evaluation of potential negative impacts represented by dollars, injuries, and barrels of oil spilled.

Minerals Management Service
Oceaneering, Inc., Houston
Shell Oil Company, Houston

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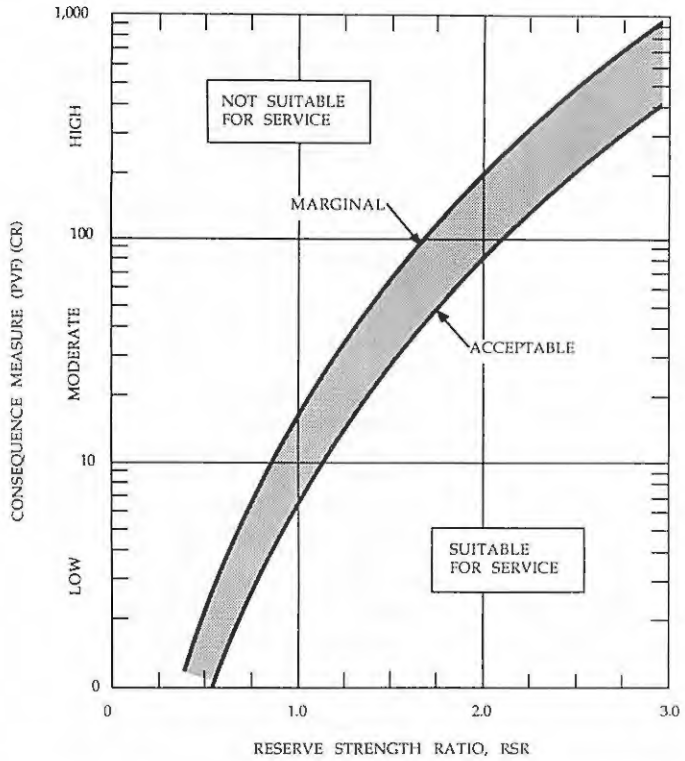

Figure 10. Suitability for service as function of consequences and RSR based on minimum total cost model.

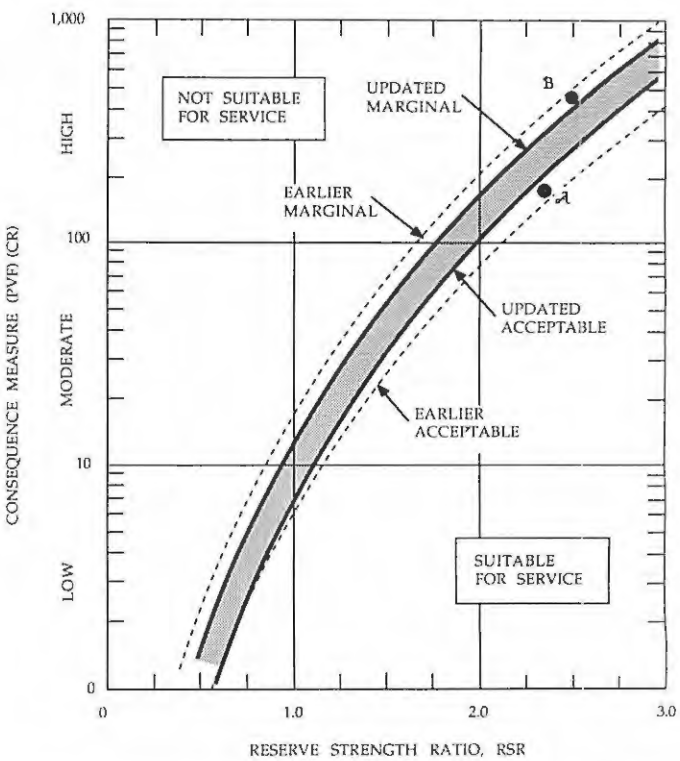

Figure 12. Influence of IMR program on acceptability criteria.

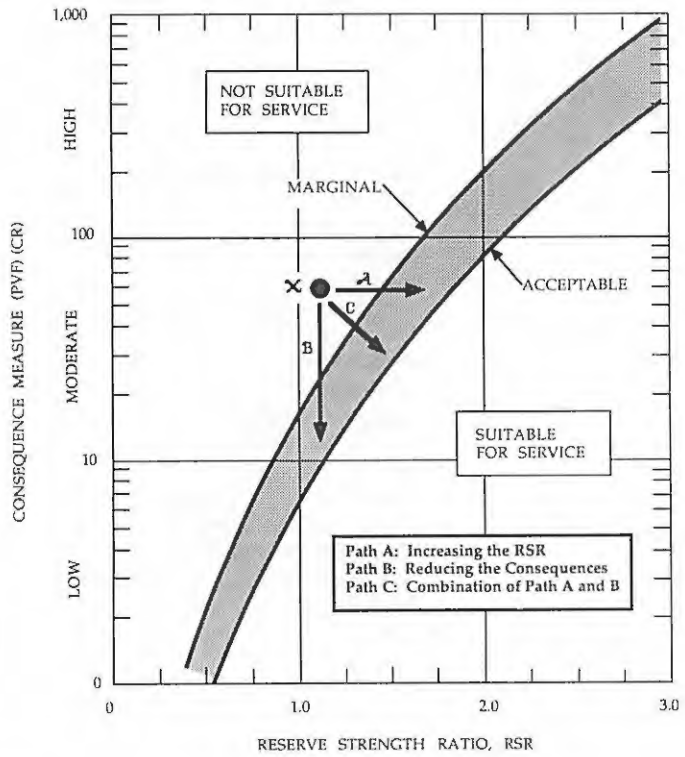

Figure 11. Alternative ways for upgrading of platforms.

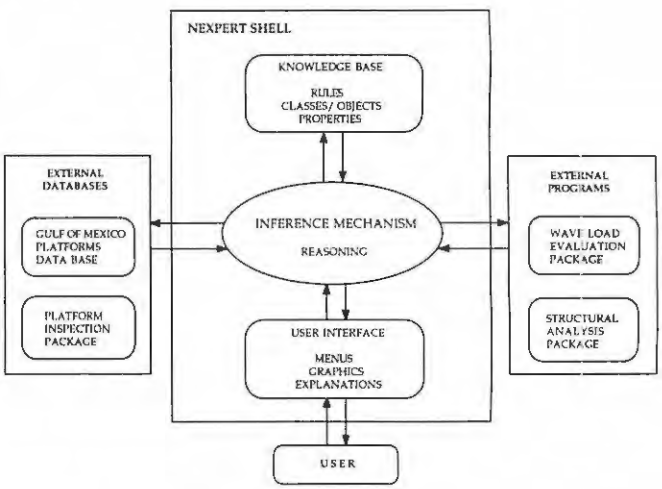

Figure 13. Organization of the expert system.

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Lecture

Sea Grant Symposium on Ageing Marine Structures, University of California, Berkeley.

Marine Affairs

Economic Values of San Francisco Bay Fisheries and Water-Quality Management

University of California, Berkeley
R/MA-29
Project Initiated: October 1, 1987
Project Completed: September 30, 1989

W. Michael Hanemann and Anthony C. Fisher

The objective of this study was to develop and study models of the impacts on the California Central Valley chinook salmon and striped bass populations associated with changes in key fisheries and variables of water-flow management. Discussion of the models is followed by a review of the main results of research aimed at measuring the economic benefits of improved harvests arising from changes in the management variables.

The Salmon Fishery

The initial year of our research on the California Central Valley chinook salmon fishery focused on the description and analysis of basic trends and underlying ecological relationships. In the past year, we have progressed to modeling the impacts of climatic variation and environmental (water flow) controls on the size and composition of the salmon population and the fishing harvest. We have made considerable progress in demonstrating the value of coordination between water-resource and fisheries-management agencies in achieving population and harvest targets.

We began the modeling process by building on our earlier work to develop very simple simulation models focused on the interaction of the hatchery and naturally reproducing populations. We were able to establish that continued dependence on hatcheries, together with the kind of fishing regulation used by the Pacific Fisheries Management Council, would tend to decrease the population of naturally reproducing fish even in the absence of environmental stress.

We then developed a more complex model of the salmon fishery that allowed us to focus on several important environmental interactions and population-dynamics phenomena. This model followed annual cohorts of fish from the number of eggs through outmigration, ocean life, and death by spawning, fishing harvest, or mortality. It allowed us, for example, to view the number of fish in the ocean in a particular year as a combination of newly outmigrated smolts, two-year-olds, three-year-olds, and four-year-olds. It separately followed the populations of hatchery and naturally reproducing fish. This model allows the user to specify annual rainfall conditions as classified by the California Department of Water Resources and then predicts the ecological relationships in the freshwater population, which depend on rainfall year type. The number of outmigrating smolts is estimated as a function of adult escapement, upstream water operations, and the position of the Delta cross-channel gates (a proxy for water exports).

Most of the key "macro" ecological relationships for the model were drawn from an extremely complex, detailed "micro" model developed by Biosystems Analysis, Inc., for the National Marine Fisheries Service and the California Resources Agency. Working with the ecologists at Biosystems Analysis, we were able to use this model to estimate the key ecological relationships in a simplified form. For each rainfall year type, we ran the model many times over the range of parameter values that were deemed feasible and realistic. We then estimated a multivariate regression that simulated the more complex model in two equations for each year. These regression equations tracked smolt survival in the complex model very closely.

The equations were incorporated into our model, along with other parameters describing survival in the San Francisco Bay and Pacific Ocean. The user can specify a string of year types and then test how various policies will affect the fishery. These policies can be constant or dependent on the values of other variables that regulators can control. This allows an examination of the value of coordinating decisions about water resource management with those affecting the fishery and vice versa.

Because of unexpected data limitations encountered by Biosystems Analysis modelers, we have so far been able to successfully model just three flow year types: wet (1975), above normal (1978), and critical (1976). We believe that these years are sufficient to make useful observations about the interaction of environmental controls and the salmon fishery.

Given the same set of environmental circumstances and controls, the number of outmigrating smolts was far higher in an above-normal year than it was in a critical year. The wet year modeled here had a smolt rate that was somewhat lower than that of an above-normal year but that was still well above that of a critical year. The model is useful for indicating how smolt output changes in different types of flow years when water-flow controls are varied. We specified that the fishing catch and the escapement of spawning salmon to the river system would be under the control of fisheries regulators. If other factors were held constant, increasing target escapement yielded the biggest increase in smolts reaching San Pablo Bay in above-normal years. In percentages, the increase was greatest in critical years. Unfortunately, however, because fishing regulators cannot predict rainfall with any reliability, this information cannot really be used to plan policy.

We were able to examine how water releases in the crucial April–May period affect survival; this may be more useful because the type of rainfall year is fairly well

known to the regulator by the time these decisions must be made. We looked at additions and subtractions to river flow in three locations: at the Red Bluff Diversion Dam on the main-stem Sacramento, at the Thermalite Dam on the Feather River, and above the Nimbus Hatchery on the American River. If escapement and operation of the cross-channel gates were held constant, changes in flow were far more important in critical years than in above-normal or wet years, in absolute as well as percentage terms. However, at higher levels of water release, the marginal increase in survival fell off for critical years.

A similar pattern holds for limiting water exports from the Delta through operation of the cross-channel gates. The increase in survival from leaving the gates closed for longer periods of time in the April-May smolt outmigration period is far greater in critical years than during other years, again in both absolute and percentage terms. In wet years, water flow is so high that our model shows almost no improvement in survival from decreased water exports during the spring outmigration.

Additional water releases or decreased exports during crucial periods in these rainfall years can have significant effects on the natural (nonhatchery) salmon population. These results imply that changing the timing of water use by hydropower and agricultural users in drought years can have important benefits for the natural population.

In critical and above-normal years, the interaction of the controls is important. Survival increases less rapidly for water additions upstream when water exports are lower. The opposite also holds: Decreased water exports have less of an effect on overall survival when upstream flows are higher. This suggests that a combination of the two controls might prove a more cost-effective way to improve environmental conditions for smolts than would total reliance on one or the other. Our model shows higher escapement levels as having much less important interactions with flow changes or water exports.

Our research shows that the size of the hatchery population trucked directly to Rio Vista or San Pablo Bay before release is of great importance. Increases in hatchery releases can greatly increase the population size and the fishing harvest. They do so at the cost of the natural population, however; at a given escapement level, increases in hatchery output cause lower spawning populations of nonhatchery fish. Since hatcheries turn away many returning adults, which then spawn in adjacent reaches, this is only a problem near hatcheries to the extent that hatchery fish spawning in the wild are regarded as different than nonhatchery fish. It is much more of a problem for populations whose spawning grounds are not contiguous to the major hatcheries.

If there is concern about population balance (natural vs. hatchery), our research suggests that, during above-normal rainfall years, hatcheries should limit their output. The natural population is very productive in these years, and limiting hatchery output will increase the percentage of returning nonhatchery adults at a cost to the fishing market that is small relative to such a cost during the critical years. Because the natural population will produce drastically fewer outmigrating smolts during critical years, the hatcheries' output is more important in keeping total population and harvests up.

If there is no concern about population balance, increases in hatchery output allow the population to be maintained with far lower spawning escapement and correspondingly higher commercial and sport harvests. Our research suggests that, if regulators believe a salmon is a salmon is a salmon, increased hatchery output and larger fishing quotas in all years may be their best policy. We should note, however, at least one reason why balance may matter: the risk of disease from relying too much on a few hatcheries.

Both in population size and the percentage of natural salmon in that population, higher escapement, higher water flows, and lower exports are always beneficial. However, they help more during critical years than during above-normal or wet years.

The Striped Bass Fishery

Our analysis of the striped bass has focused on the reestimation of statistical relationships between flow parameters and the production of the bass, as well as on the use of these estimated relationships in a simulation model of the striped bass population. Our work has not been an attempt to understand the striped bass life cycle under some strict standard of scientific accuracy as much as it has been an attempt to address certain public policy questions in the face of uncertainty. The flow of water in the San Francisco Bay/Delta Estuary is in large part controlled by upstream storage and releases and by upstream diversions, as well as by exports of water from the Delta. The impact of these flows on the Bay/Delta ecosystem has been the focus of a vigorous public policy debate. Our work is intended to shed some light on the trade-offs between various policy options to help achieve an improvement in the ecosystem at the least cost to water users.

We have examined the literature and the available data on the striped bass population in the estuary. These sources suggest that there are a number of possible explanations for the decline that has occurred over the past 20 years in both the size of the adult bass population and in the reproductive success of that population. But there does not seem to be a consensus as to the proper weight to assign to the various possible causes of the decline.

Several of the possible causes relate to the impact that water flows in the estuary have on the production and survival of young striped bass. Despite a great deal of research that has been conducted over the past 20 to 30 years, the evidence is still somewhat ambiguous. The production of young bass is governed by the complex interaction of a great many factors, most of which have a large degree of stochastic variability, so that identification of specific causes and effects is extremely difficult. If this were simply a case of scientific curiosity, then there would

be no need to resolve the ambiguity immediately. But the California State Water Resources Control Board is in the process of redefining rights to the water that might otherwise flow into the estuary and by court order must take into account the impact on the ecosystem when considering these water rights issues. Thus, the impact of flows on the striped bass population has an immediate importance in an adjudicatory process that cannot be delayed while we wait for another 30 years of data on the fish.

The California Department of Fish and Game (CDF&G) has at various times estimated certain statistical relationships between flow parameters and the production of young striped bass, as measured in the annual young-of-the-year (YOY) indices. The YOY index has been produced for 30 years and consists of two parts: the Suisun Bay index (SYOY) and the Delta index (DYOY). The theoretical literature on the striped bass strongly suggests that the factors that affect the SYOY are quite different and distinct from those that affect the DYOY index. We have reexamined the statistical work and have concluded that the data support the notion that quite different flow parameters affect these two subindices. The SYOY appears to be related to the level of outflow in the spring from the Sacramento/San Joaquin Delta into Suisun Bay, whereas the DYOY appears to be related to the level of water exports from the Delta that go to the two major canal systems: The State Water Project and the Central Valley Project.

One point of controversy in the analysis of the striped bass has been that the statistical relationships between flows and the YOY indices have lost a large degree of explanatory power in the years since the major drought of 1976–1977. Several explanations have been proposed for this change. Perhaps the simplest one is that the adult population had declined by this time to a level so low that egg production was reduced sufficiently to cause a concomitant reduction in the production of young bass, as measured by the YOY indices. Although there is no scientific consensus on this point, we have found that by adding a fecundity factor to our statistical models of the relationship between YOY production and flows substantially improves the fit. Of course, the statistical relationship does not prove that there is a causal relationship. But the public policy question of how to allocate water will not wait upon the resolution of this point. Accordingly, we have provisionally accepted the results of the statistical analysis and assumed that reduced egg production due to declining adult populations has had an impact on the YOY indices.

Having estimated models of the relationship between flows and DYOY and SYOY, we have then used a simulation model of the striped bass population in an attempt to answer some important public policy questions. If we assume that it is desirable to alter flows in the estuary in order to restore the Bay/Delta ecosystem and if we assume that the health of the striped bass population is to be used as an indicator of the health of the ecosystem, we need to know how changes in the pattern of flows will affect the striped bass. We have used a modified version of the STRIPER simulation model, as developed by Professor Lou Botsford and his associates at the University of California, Davis, to look at this issue.

In our simulations, we modeled SYOY and DYOY separately instead of simply modeling the total YOY because both the theoretical literature and the empirical data suggest that the factors that affect SYOY are distinct from the factors that affect DYOY. So, changes in flow patterns that may improve one subindex may not have any impact on the other. This fact has a clear public policy implication. Any modification in water use that is required for the purpose of improving the state of the Bay/Delta ecosystem will impose a loss on some water users. In order to minimize that sort of loss, it would be useful to know whether the striped bass population will be more readily improved by increases in Delta outflow, which should tend to increase SYOY, or by decreases in the level of exports, which should tend to increase DYOY. In testimony before the State Water Resources Control Board, CDF&G has tended to favor increased outflow as a means of promoting the production of striped bass, since the production of SYOY has been a more reliable indicator in recent years than has DYOY. However, our early simulation results suggest the opposite: A reduction in exports in the spring and a consequent improvement in DYOY will have a greater impact on the overall level of the striped bass population than will increased outflow. This result, however, is sensitive to the estimated relationship between the YOY indices and the population of adult bass 3 or 4 years later. Although our statistical analysis suggests that DYOY is more important to the subsequent production of adults than SYOY is, no theoretical basis of support exists for this conclusion, and thus we do not have a strong degree of confidence in the statistical result.

In addition to looking at the issue of helping the bass through changing exports and DYOY rather than by changing outflow and SYOY, there is a second policy issue that our simulation modeling can address, namely, whether the bass can be helped more readily by providing increased flows in dry years or in normal years. This issue has to be viewed in an economic framework, since presumably water is more valuable to users when it is scarce, as in dry years, and less valuable when it is in greater supply. The initial aim of this aspect of the project was to determine the relative magnitude of flow changes in dry and normal years that are required under different strategies to produce equivalent changes in the size of the striped bass population.

In our continuing analysis, we shall use the statistical and simulation models already developed to look at the possible impact of hatchery production of striped bass. Hatchery production is in its early stages, and the analysis must be considered tentative and somewhat speculative.

Economic Values

With regard to the economic valuation of the benefits associated with the improvements in the salmon and striped bass fisheries, our research has focused on (1) commercial values for salmon, (2) sportfishing values for salmon, and (3) nonuse (existence) values for salmon and striped bass. (There is no commercial fishery for the striped bass, and very little information is available about sportfishing for striped bass.)

The economic impact associated with any change in the commercial harvest of Central Valley chinook is measured in terms of the resulting change in the incomes of California households (i.e., the change in employee income plus the change in profits). In turn, this can be divided into the direct impact on personal income in the harvest sector; the indirect impact on personal income earned in other sectors closely related to the harvest sector, such as the processing and retail sectors; the indirect impact on personal income in all other sectors of the California economy; and the induced impact on personal income throughout the California economy. Our estimates of these impacts are based on the input-output model of the California commercial fishing industry in King and Flagg (1984), which we modified in two ways. First, the relevant input-output coefficients for policy analysis are marginal rather than average coefficients, whereas the data in King and Flagg's model are average coefficients. Second, the impacts of a change in the commercial harvest of Central Valley chinook may be mitigated to some degree if firms are able to substitute other activities for those associated with the harvesting of salmon—if, for example, the retailing sector responds to a reduction in the availability of salmon by selling other fish (possibly imported salmon). Our analysis is adjusted for both of these factors. Also considered is the possibility that a reduction in the availability of Central Valley chinook would lower the income of the harvest sector but that this sector would still spend as much as it did before and the only other impact on the California economy would be that induced by this reduction in harvest sector income. This analysis leads to commercial values of about $29 and $80 per fish, respectively. We believe that these two estimates probably bracket the range of possible economic impacts associated with a change in the commercial harvest of Central Valley chinook. Furthermore, a change in harvests could affect consumers through price increases and a loss of consumer's surplus. Our analysis, based on the very limited data available, suggests that this could amount to as much as $15–$30 per fish.

With regard to the sportfishing values of salmon, we reviewed the existing literature on travel cost models of salmon fishing on the West Coast but encountered conceptual and data problems that prevented extrapolating for their results to the valuation of the Central Valley chinook salmon. Accordingly, we developed a synthetic model relating the abundance of chinook to the participation in party-boat fishing for salmon in California, using annual data for the period 1976–1985. Various functional forms were analyzed, and all generated similar results. These suggest that a reduction in the total stock of 10,000 fish would generate a reduction of 1,200–1,500 days of party-boat fishing in California. Valuing a day of party-boat fishing at about $80 implies a marginal loss-of-use value of about $60 per fish reduction in the ocean harvest of Central Valley chinook.

In addition to commercial and sportfishing values, Californians may place a value on the protection of salmon and striped bass in their own right. This nonuse value can be estimated only through the method of contingent valuation. Here, we relied on two sources of information. The first is a study conducted by the National Marine Fisheries Service (NMFS) over the period 1985–1986 (Thomson and Huppert, 1987), which focused exclusively on Bay Area anglers and asked them to place a value on a 50% decrease in their current catch rates for both salmon and striped bass. While couched in terms that refer explicitly to sportfishing, there was no reason why the survey would not have picked up the nonuse values that anglers place on the preservation of these fisheries. It generated an average willingness-to-pay (WTP) of about $33 per angler and an average willingness-to-accept (WTA) of about $96 per angler (in 1986 dollars). Totaled for all Bay Area anglers and updated to current dollars, this amounts to a WTP of about $17.5 million per year and a WTP of about $43.5 million per year for a 50% reduction in the catch rates for salmon and striped bass. The second source of information is a contingent valuation study of the fish and wildlife resources of the San Joaquin Valley conducted in 1989 for the Inter-Agency San Joaquin Valley Drainage Program (Loomis et al., 1989). The survey covered over 1,000 California households drawn from the entire population, and one of the items valued was a program to increase flows in the upper San Joaquin River, downstream of Friant Dam, which would raise the population of chinook salmon returning to spawn upstream of the confluence with the Merced River from a current total of fewer than 100 fish to about 15,000 fish annually. The survey involved a discrete-choice format with a follow-up valuation question, yielding an interval rather than a single bound for WTP. Our analysis showed that the use of interval data yielded a significant gain in precision over the conventional, single-bound data. For the typical California household, our point estimate of WTP for restoring the San Joaquin River amounted to about $100 per household, or about $100 million for the entire state population. While these estimates require further analysis and refinement, they suggest strongly that the nonuse values associated with the preservation of Central Valley fisheries may be substantially larger than the use values stemming from commercial and sportfishing.

Cooperating Organizations

Environmental Protection Agency

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Global Economic Change, U.S. Foreign Policy, and the U.S. Tuna Industry Since 1949

University of California, Berkeley
R/MA-30
Project Initiated: October 1, 1988
Project Completed: September 30, 1990

Harry N. Scheiber

This project has investigated the relationship of global economic change and U.S. foreign policy to the American tuna industry since 1949. The research is an analysis of the policy process with respect to marine fisheries, ocean law and policy, and international trade policy. A major focus is the assessment of the various responses of the American industry to market forces and to changing U.S. policies in the last 40 years. It is planned that the project results, some of which (e.g., Scheiber, 1990a, 1990b) have already been presented in the United States and abroad in papers, panel contributions, and published articles, will become part of a book-length monograph on these issues.

The project leader conducted research in archival materials gathered at the University of Michigan manuscripts library, the Gerald R. Ford Library, the U.S. National Archives in Washington, D.C., and the National Archives of Canada in Ottawa. The collection, survey, and analysis of these materials continued throughout the period of the project. The data have been integrated (1) with complementary documentation photocopied and arranged by the trainee from the American Tunaboat Association Papers held in the San Diego State University Library and (2) with data collected by the project leader from the California Academy of Sciences collections of Wilbert M. Chapman and Robert Miller letters and documents; from the Milner Schaefer, Roger Revelle, Subject File, and Directors' Files collections of the Scripps Institution of Oceanography Archives, La Jolla; and from the Hawaii State Archives and Pacific and Hawaii Collection, University of Hawaii Library. All the foregoing have a bearing on the policy process in relation to the post-1949 development of Pacific tuna fisheries and on the responses to market changes and policy initiatives of the American Tunaboat Association, the Tuna Research Council, other organizations, the trade press, and individual spokesmen for tuna fishing and canning interests.

Topics that have been investigated include the major causal relationships in the dynamics of the global tuna fisheries and market expansion in the period of this study. The main elements of the industrial-sectors portion of this study include a chronology, narrative, and analysis of Japanese policies for expansion of the distant-water tuna fleet, for the coordination and promotion of exports to the United States and other foreign markets, for the resolution of intraindustry conflicts on tuna expansion and export control, and for overseas investments in onshore facilities and joint ventures. Similar data on the American distant-water tuna fleet have been collected and analyzed also. This element of the study concentrates on responses to the inflow of Japanese tuna products after 1948, to dumping and other marketing tactics that threatened the stability of the U.S. market and the competitive position and earnings of the U.S. fleet, and to American tariff and foreign trade policies that had a direct impact on the structure of the American tuna markets.

A second major area of investigation has been the policy process and the substance of policies that had a major impact on the U.S. distant-water fisheries for tuna. These include both the national policies on trade and the U.S. Government's positions on the U.S.-Japanese relationship generally; the programs for research and development, including the long-term program of the National Marine Fisheries Center Honolulu Laboratory, and state and industry efforts, such as the Hawaii initiative that resulted in creation of the Pacific Islands Development Commission and successor efforts; and the state programs in California, including the scientific programs based at Scripps Institution of Oceanography.

A third area of inquiry is the management of Pacific tuna undertaken through the Inter-American Tropical Tuna Commission, beginning in the 1960s (building on a decade of previous research) and continuing thereafter, including its impact on Japanese, American, and Latin American fishing fleets.

Finally, the study has collected data from diplomatic and scientific archives and policy documents on the global context of policy conflict over tuna. For the early period covered by the analysis, the project leader has searched the Canadian archives in Ottawa and the Australian National Archives as well as domestic U.S. sources and the records of international bodies, including the Far East Commission, 1949–1952. This last phase of the study considers the relationship of U.S. policy and international management initiatives to the emerging Law of the Sea and (finally) Exclusive Economic Zone policy formation. Attention is given to the development and impact of the U.S. official posture on tuna as a highly migratory species.

Research to date has provided strong support for the hypothesis that truly effective response in support of the competitiveness of the U.S. distant-water fleet has been frustrated at many junctures by the complexity (fragmentation) of interests within the U.S. industry itself and that the priorities variously given

by national policy makers to objectives in foreign policy, including both trade and oceans policy, have in many respects since 1949 worked to the interest of the domestic tuna fleet's overseas competitors, particularly Japan in the 1940s to 1960s but also including other Asian fishing powers in tuna waters since the 1960s. During the first year of the grant, the project leader also coordinated the editing of the Sho Sato Conference symposium papers, now published in the 1989 *Ecology Law Quarterly*. This includes an introductory essay (Scheiber, 1989a) and a monographic article on a major issue in Japanese-U.S.-Canadian relations during the postwar era (Scheiber, 1989b). The contributions to this symposium complement the earlier standard studies of Japan and the United States in Pacific affairs (Friedheim et al., 1984; Akaha, 1985); and they open up several important new lines of analysis, addressed either to single nations and their policies or else, in the mode of comparative analysis, to the policies of several Pacific Ocean powers.

The project leader has also presented materials drawn from the segment of this project on U.S. tuna research enterprises and their impact on policy and on the industry at the 18th International Congress of History of Science (Scheiber, 1989c); this material is being revised for publication in fuller form.

Among the works being prepared now from data collected from archival and printed materials is a collaborative working paper by the project leader and trainee on the Pacific Islands Development Commission. This study will add significantly to the analysis of Pacific Islands tuna by Doulman and others (1987a, 1987b). Also, it will complement and fill out the introductory analysis and materials on California state tuna policy from the late 1940s to the 1970s as presented in the book by McEvoy (1986), which was derived from a previous Sea Grant project directed by the project leader, and in the project leader's earlier writing on the Inter-American Tropical Tuna Commission and on tuna, law of the sea policies, and markets (Scheiber, 1986, 1988).

The project leader also served in 1989–1990 as convener and director of an international experts' conference, "Ocean Resources: Industries and Rivalries Since 1800," which was held at Berkeley under the joint auspices of the California Sea Grant College, the All-UC Intercampus Economic History Program (Office of the President), and the UC Berkeley Center for the Study of Law and Society. Most of these papers were revised for presentation at a theme session organized by the project leader at the invitation of the International Economic History Congress, held in Leuven, Belgium, in August 1990. This was the first session in more than twenty years devoted to history of ocean resources in these quadrennial sessions, and the papers are a fresh set of contributions to scholarship and policy analysis in the area of ocean studies. They have been made available for distribution in a Working Papers volume (Scheiber, 1990b).

Cooperating Organizations

Australian National Archives, Canberra
Canadian National Archives, Ottawa
Department of External Affairs, Canada
Gerald R. Ford Presidential Library, Ann Arbor, Michigan
Hawaii State Archives
Hawaii State Library
San Diego State University Library
U.S. National Archives, Washington, D.C. and Suitland, Maryland
U.S. National Marine Fisheries Service, Southwest Fisheries Center, La Jolla
University of California, San Diego, Scripps Institute of Oceanography Archives
University of California, Berkeley, Institute of International Studies
University of California, Berkeley, School of Law, Sho Sato Fund for Japanese–U.S. Legal Studies
University of Hawaii Library
University of Michigan Library
University of Washington Library, Seattle

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Scheiber, H. N., and C. Carr. 1992. In press. From the Truman Proclamation to the twelve-mile limit: Constitutional and historical perspectives. *Territorial Sea J.*

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Deterring Oil Spills: Optimal Policies

University of California, San Diego
R/MA-31
Project Initiated: October 1, 1989
Project Completed: September 30, 1990

Richard Carson, Ted Groves, and Montserrat Grau

As part of this project, we first analyzed the behavior of a firm in an environment with pollution externalities and technological progress. We assumed that firms may not purposely violate the pollution control regulations but that they nonetheless generate some pollution due to negligence. Our model allows firms two possible actions: either increase the level of treated waste or pay an expected penalty if illegal pollution is detected. The results show that in a world with pollution externalities, technological progress does not guarantee increases in the welfare level. Most important for policy purposes, our analysis shows the trade-off between the policy instruments: penalties, taxes, and treatment cost in a world where technological progress occurs and firms may violate the law.

Secondly, we model the occurrence of an oil spill as a stochastic event. No shipowner or oil-carrying firm chooses the size of the spill. Characteristics of the ship, and the different types of operating environments determine a stochastic process governing the time patterns and size of spills. Both the time distribution of different types of oil spills, and the distribuiton of spill size are affected by pollution control instruments such as fines, by enforcement effort, and by the diligence of ship personnel. The stochastic model allows us to see how each step of the spilling process is affected by each policy measure and to compare the relative efficiency of different measures in reducing spills.

Lastly, we estimate the parameters that govern oil spill frequency and size distribution. We model how these parameters depend on two pollution prevention measures: monitoring of transfer operations and assessment of penalties. We show that these measures reduce the frequency of oil spills. But we also show that the policy measures studied were almost irrelevant to the size of the spills.

Publications

Grau, M. V. 1991. Monitoring and pollution control: A stochastic process approach to model oil spills. Doctoral dissertation, Department of Economics, University of California, San Diego.

Rapid Response

Measuring Overwash on a Barrier Island

University of California, San Diego
R/NP-1-17A
Project Initiated: October 1, 1987
Project Completed: September 30, 1990

Robert T. Guza

Overwash of the Louisiana barrier islands during winter storms is thought to contribute significantly to the islands' rapid erosion and landward migration. The goal of this project was to monitor overwash events at Isles Dernieres, Louisiana, and determine their importance to sediment transport and morphological change.

An extensive array of instruments (three electromagnetic current meters, about 20 pressure sensors, three thermistors, and a run-up meter) were deployed at Isles Dernieres, February 12–27 1989, and a reduced array from February 28, 1989, to March 18, 1989. These instruments were an integral part of a large experiment which included investigators from the U.S. Geological Survey and Louisiana State University. The instruments functioned well. Unfortunately, no overwash events or storms were encountered. Such storms usually occur at least once a week at the time of year the instruments were deployed. This was the only winter season in recent memory during which no overwash events occurred. The experiment basically failed because of calm weather. Despite these difficulties, the data set is of interest for the study of nonstorm conditions. In particular, an extensive data set of run-up was obtained concurrently with detailed measurements of the beach-face elevation profile. These data are being used in ongoing studies of swash dynamics and changes in beach-face morphology during relatively calm weather.

Cooperating Organizations
United States Geological Survey

Publications
Guza, R. T., M. C. Clifton, and W. A. Boyd. 1988. Measuring overwash on a barrier island. *EOS Trans. AGU* 69:1238. Abstract.

Selection of Sites for Bivalve Bioindicator Monitoring Programs

San Francisco State University
R/NP-1-17L
Project Initiated: May 1, 1988
Project Completed: September 30, 1989

Douglas A. Segar

This study has led to the establishment of several criteria for the selection of sites for the Status and Trends Mussel Watch Program of the National Oceanic and Atmospheric Administration (NOAA). Also, a new approach to trends determination has been developed and tested; it involves spatial bulking of data from several adjacent sites within a geographically distinct cluster and leads to a substantial improvement in the statistical power to detect small temporal changes in mean contaminant concentrations within defined areas of the coastal zone. This improvement in the sensitivity to detect trends now can be achieved without substantial increases in sampling or cost.

The steps needed to ensure the proper evolution of the Mussel Watch project and the appropriate modification of the existing site grid are indicated in the following summary of findings.

Status assessment requires more sites than does trends assessment. An appropriate number of trends sites should be selected in clusters, each of which represents an area (e.g., defined region, bay, estuary, location within pollution gradient) within which temporal trends of contamination should be measured. Areas to be monitored should be carefully selected. The temptation to reduce the number of sites within a cluster in order to monitor more areas must be avoided, since this will compromise the success of the trend-monitoring program in all areas monitored. Trend-monitoring sites should be sampled each year.

Status-monitoring sites should be selected to periodically establish (1) the status of contamination of areas of the coastal oceans, estuaries, and embayments that are not monitored by trend sites and (2) the detailed distribution or status of contamination gradients in areas contiguous to trend-site clusters or between sites in clusters with widely separated sites.

General criteria were identified that apply to all status and trends sites. These criteria include a stable population of the chosen bioindicator organisms within the selected site; accessibility of the site; avoidance of areas potentially contaminated by nearby point sources, artificial structure substrates, or other local sources; and sites at which sampling can be done within a defined depth range on similar substrates.

Additional criteria that apply to status sites were identified. These address factors such as the need to fully characterize pollution gradients and identify all regions of contamination that are larger than a defined minimum size; and the need to establish appropriate background sites. Also determined was that status sites do not need to be sampled more often than every few years.

Criteria applied to the selection of trends-assessment sites address the following needs: use of site clustering (spatial bulking) of several sites within the same part of a pollution gradient to improve the power of detection of temporal trends and to reduce the number of analyses, both small-scale geographic and time-by-location variance; minimizing of variance due to small-scale temporal changes in pollutant loading by avoiding the sampling of areas too close to pollution sources; use of larger sites to ensure better characterization of small-scale geographic variance; sampling of each site annually by taking at least three separate replicate samples per site; selection of site clusters within contiguously mixed regions of a bay, estuary, or ocean; location of all sites in a cluster within approximately the same salinity regime; sampling of the same species at all sites within a given cluster; establishment of open-coastline site clusters that are widely spaced in order to establish regional background-contamination changes; selection of at least four sites per cluster; and continuous reevaluation of the appropriateness of each site for inclusion in a cluster.

Sites for trend-site clusters should be selected through detailed analyses of existing data, reconnaissance surveys of pollutant concentrations in bivalves from 10 to 20 sites within each area (pollution gradient, uncontaminated coastline, bay, or estuary) for which trend monitoring is performed; and detailed studies of the known pollutant sources and local hydrodynamics in each area.

In order to implement the redesign of the Status and Trends Mussel Watch Program most effectively, existing sites should continue to be monitored annually, and trends should be monitored through both individual site data and data from existing viable site clusters. Each year, a specific section of the U.S. coastline should be sampled more extensively than other segments to identify effective locations of sites and site clusters for trend assessment in predetermined areas of interest. Only those sites selected for inclusion in trend-site clusters should be resampled annually in this section of the U.S. coastal zone after new site clusters have been established. If the trends objective is properly fulfilled in critical parts of each region, a 5- or 6-year frequency for status determination would be acceptable.

Cooperating Organizations
National Oceanic and Atmospheric Administration, National Status and Trends Program

On-Board Handling of Albacore Tuna for Alternative Markets

University of California, Davis
R/NP-1-18A
Project Initiated: January 1, 1989
Project Completed: March 31, 1989

Robert J. Price and Edward F. Melvin

The overall objective of this project was to improve the quality of albacore landed for use in alternative, noncannery markets. Specific objectives were to determine the shelf life of fresh and frozen albacore under controlled handling and storage conditions, and to determine the effects of handling techniques, freezing methods, and storage conditions on albacore quality. From the data obtained, recommendations were developed concerning handling practices and freezing methods for use by the industry in developing alternative markets for fresh and frozen albacore.

Albacore were captured on commercial fishing vessels; killed by brain destruction; and dressed, bled, or left round. Seawater temperature at capture averaged 62° ± 1°F. Albacore backbone temperature immediately after capture ranged from 75.0° to 91.7°F and averaged 85.4° ± 3.5°F.

Albacore for fresh studies were chilled to 32°–55°F in a seawater/ice slurry and transferred to storage in ice. Albacore were held at 32°F and sampled at 3- to 4-day intervals for 33 days. Samples were examined for aerobic plate count (APC), pH, histamine, trimethylamine nitrogen (TMA-N), and nucleotide content.

Chilling albacore in a seawater/ice slurry was effective in reducing flesh temperature rapidly. Iced storage of albacore produced excellent-quality fish with a long shelf life. Albacore flesh pH increased from 5.7 to 5.9 during storage, and APC increased slowly and reached about 10^7/g at day 16. Histamine (65.9 ± 23.5 mg/100 g) was detected only after 33 days. The TMA-N content increased gradually, reaching 2.6 ± 0.1 mg/100 g after 33 days. Of the nucleotides, adenosine triphosphate (ATP) and adenosine diphosphate (ADP) were not detected at day 1, inosine monophosphate (IMP) decreased linearly with time, and hypoxanthine (Hx) was produced slowly during storage. IMP and ratios of nucleotides appear to be good indexes of iced storage life.

An estimated shelf life for iced albacore is about 11–14 days for high quality and 16–21 days for acceptable quality; after 26–28 days, quality is unacceptable. This study provided no evidence that bleeding or dressing albacore before chilling had any effect on the quality or shelf life of the iced fish. Dressed albacore cooled more rapidly and had better belly-cavity appearance than round or bled albacore. Rapid chilling and good temperature control during storage are the most important factors in maintaining the quality of fresh albacore during iced storage.

Albacore for brine and brine/coil frozen storage studies were chilled in a saturated brine solution at 10°F until the backbone temperature reached 28-34°F. Brine/coil-treated fish were transferred to dry storage at 20°F, and brine-treated fish were left in saturated brine at 10°F for 3 weeks to simulate storage at sea and then transferred to dry storage at –12°F to simulate storage ashore for a total of 12 months of storage. Albacore for blast-frozen studies were frozen in a blast freezer at 0°–5°F, stored at 10° F for 3 weeks to simulate storage at sea, and transferred to dry storage at –12°F to simulate typical storage ashore (total storage 12 months). After 3 weeks at 10°F, albacore steaks from each treatment were vacuum-packaged to investigate the frozen shelf life of vacuum-packaged albacore steaks.

Chilling of albacore in a saturated brine solution at 10°F was effective in reducing flesh temperature rapidly. Albacore were sampled at 3-month intervals for APC, flesh pH, salt, dimethylamine nitrogen (DMA-N), thiobarbituric acid–reactive substances (TBARS), and nucleotide content. After 9 months, frozen, vacuum-packaged albacore steaks were evaluated to determine if the general population could determine sensory differences between round, bled, or dressed albacore and a high-quality-control albacore.

During 12 months of frozen storage, APC remained between 1.3 x 10^2 and 2.7 x 10^3/g, and flesh pH values increased slightly from 5.73 to 5.87. Brine-frozen and brine/coil-frozen albacore generally had a higher salt content than blast-frozen albacore. No significant differences were found in the salt content of round, bled, or dressed fish or in the salt content of vacuum-packaged steaks versus unpackaged fish at 12 months within each freezing group (brine-, brine/coil-, and blast-frozen). After 9 months of frozen storage, albacore that were cooked, bled, and dressed were significantly lighter in color intensity than were cooked, round albacore. Except for the difference in the appearance of the cooked flesh, there was no evidence that bleeding or dressing albacore before freezing had any effect on the quality or shelf life of the frozen fish different from the effects of the other variables measured in this study. No significant differences were evident between the chemical and biochemical indexes of quality for albacore frozen round, bled, or dressed and those indexes for vacuum-packaged albacore steaks. The brine-, brine/coil-, and blast-freezer systems used in this study can produce high-quality frozen albacore if the albacore are handled rapidly and frozen quickly. Based on the low DMA-N content in frozen albacore after 12 months at –12°F, the high-quality shelf life is at least 12 months.

Albacore to be frozen at sea should be stunned or killed immediately on capture to prevent damage to the flesh from struggling on the deck. Bleeding or dressing the albacore improves the

appearance of the flesh after cooking and provides an obvious sign to the buyer that the fish was bled. Dressing the albacore also removes gut bacteria and enzymes that can increase the rate of quality loss and cause discoloration and softening of the flesh near the belly, especially if the albacore are subjected to temperature above freezing.

High-quality brine- and brine/coil-frozen albacore can be produced by freezing the albacore immediately after capture in a saturated brine solution at 10°F. Brine/coil-frozen albacore should remain in the brine until their backbone temperatures are below 30°F and should then be transferred to a separate dry storage area at 10°F. Maximum on-board frozen storage should not exceed 30 days.

High-quality blast-frozen albacore can be produced by freezing the albacore immediately after capture in a blast freezer at 0°F. Maximum on-board frozen storage should not exceed 30 days.

Cooperating Organizations
Commercial albacore fishermen
National Marine Fisheries Service

Publications
Price, R. J., and E. F. Melvin. 1989. On-board handling of albacore tuna for alternative markets. Final report for U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Price, R. J., E. F. Melvin, and J. W. Bell. 1991. Postmortem changes in chilled round, bled and dressed albacore. *J. Food Sci.* 56(2):318–321.

Price, R. J., E. F. Melvin, and J. W. Bell. 1992. Postmortem changes in blast, brine and brine-coil frozen albacore. *J. Aquatic Food Prod. Tech.* 1(1):67–84.

Functional Morphology of the Lateral-Line System in Two Species of Commercially Important California Flatfishes: Ontogeny and Asymmetry

University of California, San Diego
R/NP-1-18B
Project Initiated: December 1, 1988
Project Completed: March 31, 1990

Jacqueline F. Webb

The flatfishes (Order Pleuronectiformes) are characterized by the migration of one of the eyes to the other side of the head during a unique metamorphosis that takes place before the transformation of the larva into a juvenile fish.

Postmetamorphic flatfishes lie on the blind (abocular) side of the body and generally take up a benthic existence, although many flatfishes swim actively. As a result, the axis of body symmetry common to all vertebrates lies parallel to the boundary between the benthos and the water column. This 90° rotation in body position is a secondary condition in fishes; the organ systems that exhibit bilateral symmetry in other fishes also are largely symmetrical in flatfishes.

The mechanosensory lateral-line system, a hair-cell based, placode-derived mechanosensory system, is one of these bilaterally symmetric systems in fishes. The functional unit of the system is the neuromast, which is composed of directionally sensitive hair cells and covered by a gelatinous cupula. Neuromasts are located on the epithelium and in canals (which are often bony) on the head, trunk, and tail of all fishes (Coombs et al., 1988). The system functions in the detection of prey, avoidance of predators, maintenance of position in schools, and the detection of hydrodynamic fields (Bleckmann, 1986; Hassan, 1989).

The behavioral role of the lateral-line system has been actively studied, but the morphological diversity of the system has not been fully investigated (Coombs et al., 1988; Webb, 1989a). The morphological diversity of the lateral-line system in fishes has been explored, and a limited set of defined morphological patterns of the lateral-line system of the head and trunk have been described (Coombs et al., 1988; Webb, 1989a). Further experimental work has established the fact that morphological diversity in the system has distinct functional correlates (Denton and Gray, 1988, 1989).

In flatfishes, only the portion of the lateral-line system between the eyes is modified, directly because of the migration of one eye to the other side of the head. In addition, because the two sides of the head of flatfishes face two distinct environments (the benthos and the water column) and because the system is essentially duplicated along this boundary owing to its bilateral symmetry, the opportunity exists for specialization of the lateral-line canals and neuromasts on one or both sides of the head.

Results and Discussion

This study has described the extreme in asymmetry of the lateral-line system in flatfishes that occurs in *Glyptocephalus zachirus*. It had been reported that the blind side of the head in the deep-water pleuronectid *Glyptocephalus zachirus* is characterized by mucous pits of unknown origin and function (Norman, 1934; Allen, 1982). These mucous pits have been positively identified as a set of widened lateral-line canals containing extraordinarily large neuromasts and covered by a scale-covered epithelial tympanum. In contrast, the eyed side of the head has a simple, narrow set of canals. The width of the canals containing these neuromasts differs by one order of magnitude, and there is a difference of more than two orders of magnitude between the size of the neuromasts in the canals of the eyed and blind sides of the head (2 mm vs. 50 μm). This extreme asymmetry extends to the peripheral nerves that innervate the neuromasts (branches of the anterior lateral-line nerve) and to the primary sensory center in the brain (medial octavolateralis nucleus of the medulla oblongata). This is the first example of dimorphic asymmetry within an individual (Webb, 1988). Additionally, the detailed analysis of the size of lateral-line nerve fibers and their termination in the medulla oblongata of the brain will play a very important role in the interpretation of form-function relationships in sensory systems of vertebrates.

Glyptocephalus zachirus has been a perfect species in which to begin the study of the role of heterochrony in the diversification of the lateral-line system because it has a dimorphic lateral-line system (Webb, 1988). This analysis has required the accumulation and examination of additional specimens across a wide size range, and this aspect of the project is still in progress. The divergence of the morphology of the lateral line resulting in a widened canal system on the blind side and a narrow canal system on the eyed side begins very early during larval development, well before metamorphosis. This process cannot be the simple result of the alteration of the rate of canal formation, but it must involve changes in the rate of neuromast growth and the course of ossification of the canals. Because narrow canal systems and widened canal systems otherwise occur in different species (Webb, 1989a), the investigation of ontogeny in *Glyptocephalus* will serve as a valuable model for understanding the evolutionary and functional diversification of the lateral-line system in fishes in general.

In flatfishes, the lateral-line system starts out as a bilaterally symmetric system in larvae and is modified

because of the migration of the eye and additional functional specializations. The divergence of the morphology of the lateral-line systems of the two sides of the head associated with functional specializations may be due to heterochrony, the alteration of developmental rates, which has been shown to be a major mechanism of evolution in many systems and in many taxa (Alberch, 1982). Scanning electron microscopic analysis of a laboratory-raised growth series of the California halibut, *Paralichthys californicus* revealed, however, that—unlike the lateral-line system of *Glyptocephalus*— the system of the halibut shows few if any type II specializations. So, while our original goal (to use these specimens for an analysis of heterochrony) could not be achieved, these specimens were used to address basic questions pertaining to the process of eye migration, a process unique to and universal in the flatfishes.

The data gathered for this study were generated using scanning electron microscope preparations of whole larval and juvenile fishes, and sets of histological slides prepared from specimens collected in the San Diego area. Unlike most experimental studies, the study of morphology generally involves the generation of a *permanent* data base. I have gathered hundreds of slides containing serial cross sections of the heads of eight species of flatfishes belonging to the following genera: *Glyptocephalus, Paralichthys, Citharichthys, Pleuronichthys, Parophrys, Microstomus*, and *Symphurus*. To my knowledge, this data base is unique and has provided valuable comparative data for study of the lateral-line system. In the future, it will allow the continous generation of valuable data applicable to many areas of functional morphology, ecomorphology, and development of the sensory systems of flatfishes well beyond the duration of my Sea Grant funding.

One example of the multipurpose nature of such a data base was my incidental discovery of a median, accessory olfactory sac in the California tongue sole, *Symphurus atricauda*. The nasal sacs of flatfishes do not migrate along with the eye. Flatfishes that are truly benthic (the soles) and bury themselves in sediment must be able to ventilate their olfactory sacs, especially the sac of the blind side, which is in contact with sediment that may be quite muddy and detrimental to the sensory epithelium. The examination of serial sections of the head of *Symphurus* has revealed that the two olfactory sacs that bear the sensory epithelia and that are open to the outside via incurrent and excurrent nares are joined medially by a nonsensory, epithelial sac. This sac is elongated caudally and lies beneath the medial bone of the cranium and above the mouth. Further examination of representative flatfishes in each of the three genera in the family Cynoglossidae (*Symphurus, Cynoglossus*, and *Paraplagusia*); two additional lineages of soleoid flatfishes (*Solea* and *Trinectes*); and eight Pacific pleuronectoid genera (*Pleuronichthys, Paralichthys, Microstomus, Glyptocephalus, Lyopsetta, Platichthys, Parophrys, Ammotretis*) revealed that (1) accessory olfactory sacs are present in all flatfishes examined and probably in all flatfishes (2) all pleuronectoid flatfishes examined have one or two pairs of bilaterally arranged accessory nasal sacs while soleoid flatfishes (with the exception of *Trinectes*) have accessory nasal sacs that are fused medially ventral to the parasphenoid and dorsal to the buccal cavity, and (4) this unique configuration of accessory nasal sacs is therefore a characteristic that unites the Cynoglossidae (synapomorphy) and may unite the soleoid fishes. It is hypothesized that the soleoid flatfishes use a unique mechanism of nasal ventilation in which pressure fluctuations that occur during cyclic respiratory ventilation are transmitted through the dorsal wall of the buccal cavity into the accessory nasal sac; these fluctuations cause expansion and contraction of the nasal sacs and the ventilation of the sensory nasal sacs that facilitates the acquisition of olfactory stimuli (Webb, 1989b). This is thus a testable hypothesis that may further reveal the importance of olfaction in flatfishes.

Significance of This Study

The assessment of the sensory capabilities and, therefore, of the behavioral and ecological potential of a species depends on a clear understanding of the functional morphology of the sensory systems with which it is endowed. The presence of specialized morphology in peripheral sensory systems may be an indication that the animal has modified its ability to influence the rate of stimulus acquisition (e.g., nasal ventilation) and to amplify and filter potential stimuli (e.g., lateral-line modifications), indicating the importance of these sensory systems. While visual capability has often been treated as an indication of potential behavioral capabilities in fishes (prey capture, predator recognition, and avoidance), a closer examination of the other sensory systems present in all fishes by means of modern histological and neuroanatomical methods and in conjunction with an evaluation of ecological and behavioral data will continue to be a rich source of information on the biology and ecology of fishes, with applications in fisheries and habitat management.

Cooperating Organizations

Australian Museum, Sydney
Los Angeles County Museum of Natural History
National Institutes of Health
National Marine Fisheries Service, La Jolla
Occidental College

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Legal Responses to a Rising Sea Level

University of California, Berkeley
R/NP-1-18D
Project Initiated: February 1, 1989
Project Completed: July 30, 1989

David Caron

This project had three major objectives. Two of these objectives involved legal responses to a rising sea level, and two related studies were prepared: one on international legal responses and one on U.S. legal responses. The third objective was the organization and running of an ocean resources program for the Ocean Resources Committee of the Western Legislative Conference.

The Ocean Resources Program was held in Monterey, California in November 1989; approximately 75 legislators and ocean resources specialists attended. The program covered questions of the federal and state division of ocean resources, ocean management, the history of fishing relations between Japan and the United States, and current issues regarding driftnetting.

A lecture on international legal responses (Caron, 1989) was presented both at the Monterey conference and at a December 1989 meeting of the Association of Pacific Island Legislators on the island state of Pohnpei, Federated States of Micronesia. An article (Caron, 1990) based on these lectures was published in 1990 in a law journal.

This paper on the law of baselines and a rising sea level is the first to consider the impact that a rising sea level will have on maritime boundaries. It is particularly noteworthy for its identification of the existence of legal feedback to climate change and its proposals for changes in the law of baselines to avoid aggravation of the suffering that will accompany climate change.

The Ocean Resources Program was particularly significant for its education of legislators on numerous ocean resource questions; the establishment of connections between the academic and legislative communities; and its bringing together of federal, state, and foreign officials on the question of driftnetting.

Cooperating Organizations
Association of Pacific Island Legislatures
The Japanese government
Western Legislative Conference

Reference
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Lecture
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Development of a Fish Assay for Detection of Worm Infections

University of California, San Francisco
R/NP-1-18E
Project Initiated: March 1, 1989
Project Completed: December 30, 1989

Judy A. Sakanari

Anisakiasis is caused by the accidental ingestion of the larval stage of *Anisakis* present in raw or improperly prepared fish and squid dishes. Humans can be infected by eating seafood dishes such as sushi, sashimi, pickled herring, ceviche, lomi-lomi and undercooked fish (Oshima, 1972; Sakanari and McKerrow, 1989).

Adult worms are normally found in the stomachs of seals, dolphins, and whales. Eggs from adult worms are eliminated from the host's intestine into the ocean. Larvae hatch from the eggs and are eaten by crustaceans such as krill. Infected crustaceans are then eaten by fish. In the United States, salmon and rockfish are the most common fish implicated in the transmission of anisakiasis. The life cycle is completed when the marine mammal eats the infected fish.

Symptoms of anisakiasis may be vague and vary from nausea, vomiting, and diarrhea to acute abdominal pain. Because these symptoms mimic other diseases, accurate diagnoses are often difficult. A clinicopathological study showed that over 60% of the anisakiasis cases were preoperatively misdiagnosed, as either appendicitis, gastric tumor, cancer, tuberculosis, peritonitis, or ileitis (Yokogawa and Yoshimura, 1967).

Anisakiasis has been recorded in the United States, the Netherlands, the United Kingdom, New Zealand, France, Germany, Chile, Taiwan and Japan. In Japan, where the consumption of raw fish is an integral part of the Japanese diet, there are over 1,000 confirmed cases of anisakiasis each year, and the number of undocumented or misdiagnosed cases is not known (Oshima and Kliks, 1987). In the San Francisco Bay area, my colleagues and I confirmed several new cases of anisakiasis in 1989 (Sakanari and McKerrow, 1989), and the number of reported cases is increasing and will probably continue to increase (McKerrow et al., 1988). Recently, public concern has been growing about this parasite; media coverage has included newspapers (*Wall Street Journal, New York Times, Los Angeles Times, San Francisco Examiner, USA Today*) and radio broadcasts (National Public Radio).

Several studies have been conducted to determine the survival of anisakid nematodes in herring (*Clupea harengus pallasi*), rockfishes (*Sebastes* spp.), and salmon (*Oncorhynchus* spp.) after freezing or cooking (Hauck, 1977; Deardorff and Throm, 1988; Sakanari and McKerrow, 1989). On August 21, 1987, the Food and Drug Administration (FDA) released the following Code interpretation: "Fishery products which are not cooked throughout at 140°F (60°C) or above, must have been or must, before service or sale in ready-to-eat form, be blast frozen to -31°F (-35°C) or below for 15 hours or regularly frozen to –10°F (–23°C) or below for 168 hours (7 days). Records that establish that fishery products were appropriately frozen on-site must be retained by the operator for 90 days."

However, the suggested FDA guidelines are not necessarily followed; consumers want to eat fresh (not frozen) fish. Hence, it is important to identify specifically which fish species need to be treated (commercially blast frozen) and to determine which fishes are, in fact, a public health concern.

Objective of Project

Recent public concern over the possibility of becoming infected with the parasitic worm *Anisakis* caused wholesale fish distribution and the sushi bar business to drop by 50% over a 3-month period in the San Francisco Bay area (Steve Fisher, Nikko Fish, personal communication). To identify *Anisakis* infections in fish and to determine which fish species are infected with the parasite, we must develop a specific and sensitive way in which the infections can be detected. The major objective of this rapid response project was to initiate studies for the

Figure 1. Southern blot of *Anisakis* DNA hybridized with the nonradioactively labeled *Anisakis* probe. Southern blot 1 was probed with radioactively labeled (^{32}P-dCTP) probe. Southern blot 2 was probed with nonradioactively labeled probe. Lane 1, *Anisakis* DNA cut with EcoRI and Lane 2, *Anisakis* DNA cut with Hind III.

development of an assay to detect *Anisakis* infections in fish tissue. I used molecular biological techniques to (1) isolate a DNA probe that is specific for *Anisakis* DNA and not fish DNA, (2) label the probe with nonradioactive reagents for use on a Southern blot and dot-blot hybridizations, and (3) determine the sensitivity of the probe in the polymerase chain reaction (PCR).

Summary of Accomplishments

The long-term goal is to develop a specific, sensitive, easy, and safe assay that can detect *Anisakis* infections in fish fillets.

The first step toward this goal was to isolate a DNA fragment specific for *Anisakis*. An *Anisakis* DNA fragment, isolated using PCR (Sakanari et al., 1989), was radioactively labeled and used as a probe on a Southern blot. Analysis of the blot revealed that the probe was specific for *Anisakis* DNA and not rockfish (*Sebastes paucispinis*) DNA. Thus the *Anisakis* probe proved to be specific for the parasite.

Because of the health and safety hazards associated with the use of radioisotopes and radioactive materials, I explored the possibility of using nonradioactive reagents to identify *Anisakis* DNA so that personnel may use the assay even in a setting without the Radioactive Materials License given by the California Department of Health Services.

The Genius Nonradioactive DNA Labeling and Detection kit (Boehringer Mannheim Corporation, Indianapolis, Indiana) uses a nucleotide analog (digoxigenin-11-dUTP), which is incorporated into the probe in a random-priming labeling reaction. The digoxigenin-labeled probe is hybridized to immobilized DNA, detected with an antibody-enzyme conjugate (anti-digoxigenin alkaline phosphatase), and visualized by an enzyme-linked color reaction (Figure 1). *Anisakis* larvae were removed from rockfish, washed three times in phosphate-buffered saline and gentamicin (100 µg/ml), frozen in liquid nitrogen, and stored at –70°C. DNA was extracted by grinding the worms with liquid nitrogen in a mortar and pestle. The powder was resuspended in a buffer containing 150 mM EDTA, 50 mM Tris-HCl, pH 8, 1% sarkosyl, and 300 µg/ml proteinase K. The solution was centrifuged for 5 minutes at 2,000 x G, and the supernatant was RNase-A treated. The supernatant was then extracted with phenol/chloroform and ethanol precipitated. The pellet was resuspended in 1.6 ml of water, and 1.1 ml 20% polyethylene glycol 8,000 in 2.5 M NaCl was added. The mixture was incubated for 1 hour in ice water and centrifuged for 20 minutes at 8,000 x G. The pellet was washed with 70% ethanol and dissolved in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Approximately 20 µg of DNA were loaded per well.

The *Anisakis* probe was nonradioactively labeled with reagents from the labeling kit described above. Following the procedures described in the kit instructions, I random-primed 1 µg of probe. The blot was prehybridized at 68°C for 1 hour and hybridized in 5xSSC (SaH/sodium citrate), 1.5% blocking reagent #11 provided by the kit, 0.1% N-lauroylsarcosine, Na-salt, and 0.02% SDS (sodium dodecyl sulfate) at 68°C for overnight. The blot was washed twice for 5 minutes in 2xSSC and 0.1% SDS and washed twice for 15 minutes at 68°C in 0.1xSSC and 0.1% SDS. Bands on the blot were examined after overnight incubation according to the instructions in the kit, except that the blocking reagent was increased to 1.5%, and 0.05% Tween-20 was added to buffer 1.

Results of the hybridization of the Southern blot with the

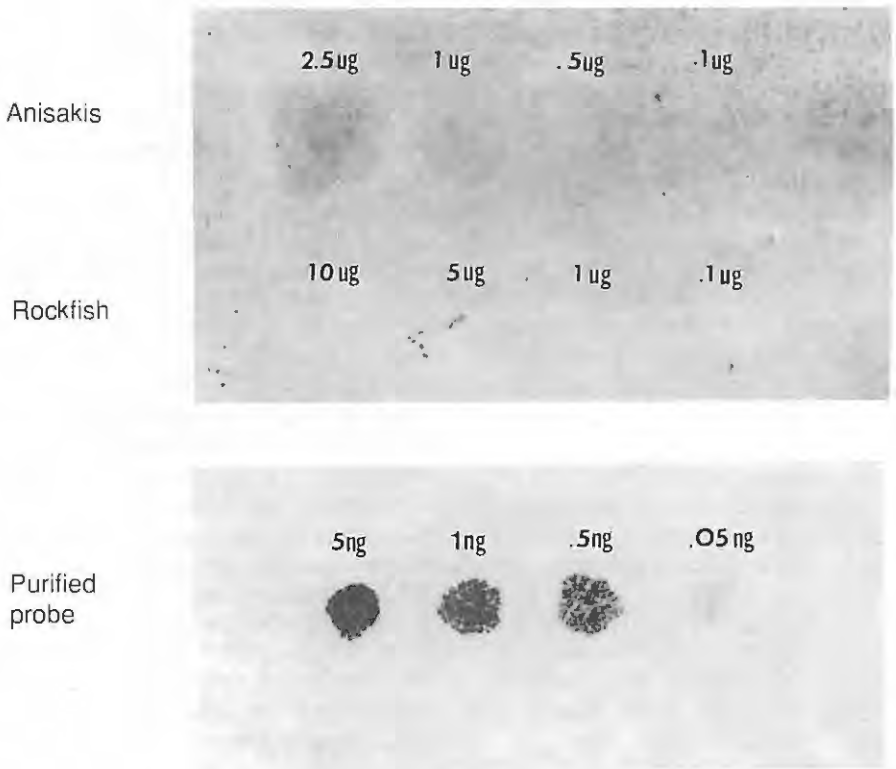

Figure 2. Dot-blot hybridization of *Anisakis* DNA and rockfish DNA using the nonradioactively labeled probe. *Anisakis* and rockfish DNA were extracted using a modification of the procedure described by Maniatis et al. (1982). Briefly, tissues were ground in a mortar and pestle with liquid nitrogen. Proteinase K (10 mg/ml) and sodium dodecyl sulfate (SDS; 20%) were added to TE (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) at final concentrations of 1 mg/ml and 2%, respectively. Samples were incubated at 42°–48°C for 2 hours and extracted with phenol/chloroform and glassmilk (Geneclean, Bio101 Inc.).

nonradioactively labeled probe were comparable to those using the ^{32}P-dCTP-labeled probe. Data also showed that this nonradioactive probe is useful after storage at -20°C for up to 4 months; in contrast, the radioactive probe has a half-life of only 2 weeks. According to the manufacturers of the reagents that I used for the labeling reaction, the nonradioactive probe should be usable for up to 1 year or more when stored in a freezer.

To develop an easy-to-use assay, I modified a DNA extraction procedure and used this DNA in dot-blot hybridizations (Figure 2). The modified procedure and dot-blots would circumvent the more labor-intensive and costly procedures of DNA preparation and Southern blotting. Results of the dot-blot hybridizations showed that (1) the probe is specific for *Anisakis* DNA and (2) as little as 1–2.5 µg of genomic DNA can be used to visualize a positive result with the nonradioactive probe.

To improve the sensitivity of the assay, I used the PCR to detect small quantities of *Anisakis* DNA. The PCR is a highly sensitive and specific technique used to detect single-copy genes in nanogram amounts of DNA. It is currently being used for molecular diagnostic purposes (for list of references, see Perkin Elmer Cetus PCR Bibliography, 1989). I used the PCR in conjunction with primers based on the exact sequence of the *Anisakis* probe (Figure 3A) to determine the sensitivity of the assay.

The PCR data showed that amplification of the target sequence can be accomplished with 150 ng of *Anisakis* genomic DNA and that the primers were specific for *Anisakis* DNA and not for rockfish DNA (Figure 3B). DNA extraction of 100 *Anisakis* larvae using a modified proteinase K/SDS extraction method yields approximately 10 µg of DNA, which is estimated to equal 100 ng of genomic DNA per worm. Based on the ethidium-bromide stained gel of the PCR (Figure 3B), it appears that a single worm can be detected. In this assay, the PCR was at least 1,000-fold more sensitive in detecting the *Anisakis* gene than was the dot-blot hybridization. The technique of gene amplification using PCR meets the criteria of being a specific, sensitive, safe, and relatively easy method by which *Anisakis* can be detected.

In conclusion, I initiated studies to develop an assay to detect *Anisakis* in fish tissues using a molecular biological approach. A specific probe for *Anisakis* was nonradioactively labeled and used in dot-blot hybridizations. Primers based on the *Anisakis* probe were used in the PCR; as little as 150 ng of genomic DNA showed a positive result. This suggests that a single-worm infection can be detected and that this approach may be useful in detecting other infectious organisms such as bacteria, viruses, and protozoa that contaminate food and food products.

Figure 3A. Sequence of the two *Anisakis* primers used in the PCR.
Anti-sense primer:
5′ ATA GAA TTC CAA ATT ATA ACC CCA CGA 3′
Sense primer:
5′ ATA AAG CTT TGC AAT AAT GCA TTG CGT 3′

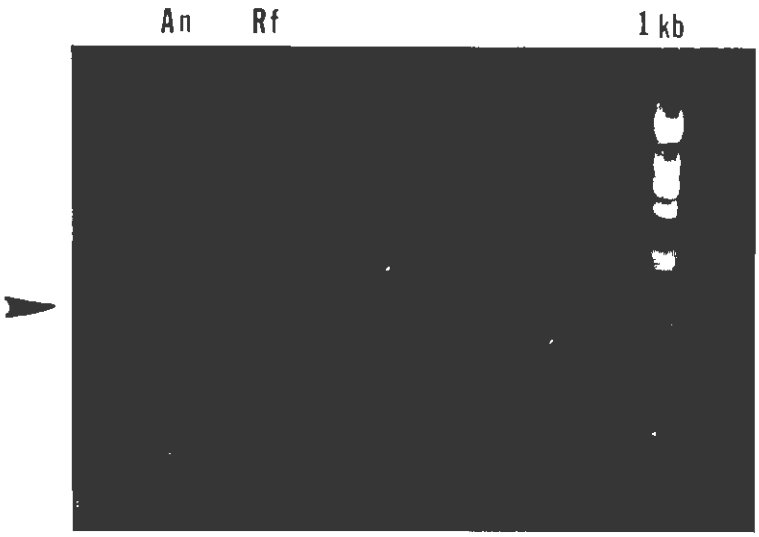

Figure 3B. Ethidium-bromide-stained gel (1% agarose) of the amplified products. One hundred fifty nanograms of *Anisakis* and rockfish genomic DNA (extraction procedure described in legend of Figure 2) were used in 50 µl reactions. DNA was amplified in the PCR as described by Sakanari et al. (1989) except that primers were annealed at 55°C for 45 cycles. Samples were run in duplicate and no DNA in the reactions was the negative control. Positive bands (150 bp) are amplified products of *Anisakis* (An). Rf = rockfish; 1 kb = standard markers.

Cooperating Organizations
California Department of Fish and Game
Hayes Street Grill Restaurant, San Francisco
Monterey Fish Company, San Francisco
National Marine Fisheries Service, Tiburon, California
Nikko Fish Company, San Francisco

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Sakanari, J. A. *Anisakis*. Invited lecture at Department of Nematology, University of California, Riverside, February 1990.

Global Warming and Upwelling Ecosystems

University of California, Berkeley
R/NP-1-18F
Project Initiated: September 1, 1989
Project Completed: December 31, 1989

Margaret S. Torn*

The objective of this project was to explore the potential impacts of global warming on California coastal ecosystems, and in particular to explore the importance of geospheric processes in linking climatic change to ecosystem change along the coast. Coastal upwelling and associated ecosystems were chosen as a case study because upwelling is a physical process with importance for both terrestrial and marine ecology. The importance of climate-driven changes in upwelling, for marine and terrestrial ecosystems as well as for people, is amply demonstrated by the El Niño phenomenon.

The climate and biota of each ocean's eastern boundary current region, such as coastal California and Peru, are strongly influenced by nearshore upwelling. These regions are characterized by highly productive fisheries, diverse and abundant bird populations, and vegetation communities associated with frequent coastal fog. The impact of climatic change in these areas involves both the magnitude of change in upwelling and the implications of change for biota.

Coastal upwelling occurs when wind stress near shore causes surtace water to be advected offshore. The diverging stream is then replaced with water from lower layers of the ocean. Along shore winds create a stress component offshore, due to the Coriolis effect, the angle of which depends on latitude. Under quasi-equilibrium conditions, the mass (M) of water advected from the coast (and replaced by deeper water) can be approximated by this equation for Ekman Transport.

$M = T/f$, where T = along shore wind stress and f = Coriolis parameter.

Upwelling is a function both of the speed of the surface wind (with stress proportional to the windspeed squared) and the direction of the wind.

It appears likely that surface winds will change as the climate responds to an enhanced greenhouse effect, and that this will lead to changes in upwelling. As Parrish et al. (1984) have written, "the locations and seasonality of maximum offshore Ekman transport appear to be closely related to the seasonal latitudinal shifts of the atmospheric pressure systems, intensification of the large-scale pressure gradients," and other factors related to global climate. With global warming, the polar regions are expected to heat more than are tropical latitudes. This differential heating pattern would change the relative difference between high and low pressure cells that drive the tropospheric circulation of the Western Hemisphere by changing, for example, the location and strength of the Pacific High. The differential heating between land and ocean surface may also change, particularly since climate will be changing rapidly with respect to oceanic responses. As a result, the low pressure found over California's Central Valley and desert may change relative to the Pacific High. Bakun (1990) found that regional-climate anomalies of this century were correlated with varying upwelling indices in several eastern ocean boundary current regions.

Potential Impacts of Changes in Upwelling

Changes in either the magnitude of upwelling or the timing of the upwelling season may have major impacts on marine and terrestrial biodiversity. Examples of the many linkages between upwelling and biological diversity are shown in Figure 1. Water upwelled from the deeper ocean is cold and nutrient rich, which is why regions of upwelling are known for highly productive fisheries. The interruption of nutrient and cool water inputs, for example, during ENSO events (El Niño-Southern Oscillation), is linked to dramatically reduced primary productivity and high mortality of certain fish and bird species.

If the greenhouse effect weakens upwelling, interrupted nutrient flows are expected. Nutrient inputs to the surface, photic zone will be reduced by more than the reduction in volume upwelled, a greater than linear response. This is due to properties of the nutrient profile and of Ekman pumping. Concentrations of nutrients necessary for algal growth, such as nitrogen and phosphorus, increase with depth in the water column. This is why upwelled water is nutrient rich. When upwelling is reduced, the depth from which water is upwelled is also reduced, so that the water upwelled has lower concentrations of nutrients than if it were upwelled from deeper in the ocean.

Using empirical nutrient profiles for the Central California Coast 1983 (Scripps, 1984) and assuming the volume upwelled is proportional to the depth of upwelling, we calculated changes in nutrient transfer to the photic zone using *a priori* changes in upwelling. Assuming a decrease in upwelling of 25% in July, the reduction in nitrogen brought to the mixed layer is 38% and in phosphate 34%. (A decrease in upwelling of 25% translated to a 26% change in heat flux, as the temperature profile is not as steep as that of nutrients.)

Reproduction and larval recruitment of marine species are particularly sensitive to the timing of upwelling because the physical advection of surface water in the Ekman layer carries larvae far offshore, outside the "nursery" where productivity and food supplies are high (Roughgarden et al., 1988). For example, larval survivorship of hake (Pacific Whiting) is decreased if upwelling is unusually early or strong (Bailey, 1981).[1]

*John Harte was project leader.

Reductions in hake numbers could have serious consequences for certain California sea lion populations. The diet of the Farallon Marine Sanctuary sea lion population is 80% hake in the spring before the sea lions pup (Ainley et al., 1982), though it is more diverse at other times of the year (Bailey and Ainley, 1982). This is one example of the way that changes in upwelling could influence several levels of a food web.

Upwelling affects terrestrial ecosystems through food chains associated with pelagic birds and shorebirds based on marine productivity (e.g., Ainley 1980; Ainley et al., 1975), and also by facilitating the formation of dense coastal fog. The cool water advected along the ocean surface cools coastal air and gives San Francisco its famous summer air conditioning, advection fog. Fog permits drought-intolerant plants such as redwood seedlings to survive California's hot, dry summers (Veirs, 1982). The distribution of many plants and animals are limited by fog frequency. Fire, too, is influenced by fog; if upwelling persists later in the fall, it will reduce the fire potential in coastal California.[2] Fire frequency and intensity promote fire-adapted vegetation communities as well as posing a risk to human health and property. By reducing solar radiation and temperature, fog also slows the formation of the constituents of photochemical smog such as ozone—a major stress on vegetation in the San Bernadino Mountains and other parklands surrounding Los Angeles.

Modelling Potential Changes in Upwelling

Coastal upwelling is a relatively tractable oceanic process for analysis since it is determined largely by local surface winds, and thus does not require a general ocean circulation simulation.

One approach to estimating changes in upwelling caused by climatic change is to calculate an upwelling index as a function of wind for today's climate and compare this with an index calculated from wind for a future climate.

Upwelling along the west coast of the United States for the past 40 years has been estimated by Bakun (1973) and Mason and Bakun (1986) using reconstructions of surface winds based on wind and atmospheric pressure observations and simple equations describing the physical mechanisms of upwelling caused by along shore wind stress. Their analysis, which yields a relative index of upwelling in mass of water advected per time per length of coastline, makes a number of simplifying assumptions: the mass of water upwelled is equal to the mass of water advected, wind speed is uniform over the time interval for which wind data are aggregated, the coastal shelf is deeper than the Ekman layer or, roughly, the mixed layer, and we have steady state motion (equilibrium time on the order of hours).

Typically, upwelling peaks along the Pacific Coast in summer and is negative (downwelling) in the north and close to zero in the south. The onset of upwelling is later, and its magnitude lower, the farther north one looks.

To estimate upwelling under a global warming scenario, we adapted Bakun's pressure-field based approach for use with surface wind vectors provided by general circulation models (GCM). General circulation model output was deemed the most appropriate source for predictions of future winds to use as input for calculating upwelling.[3] We used output from the NASA Goddard Institute for Space Studies GCM (GISS, fine scale model, Hansen et al., 1983; Hansen et al., 1988) equilibrium climate simulations of a mid-1900s atmosphere (control) and of an atmosphere containing twice that amount of greenhouse gases (2 x CO_2) NASA GISS provided data at the finest spatial resolution of the four GCMs considered.

Upwelling indices were calculated following Bakun (1973) for eight points from Baja California to Vancouver Island, 26° to 50° N latitude, corresponding to the midpoint of eight 4° x 5° GISS GCM

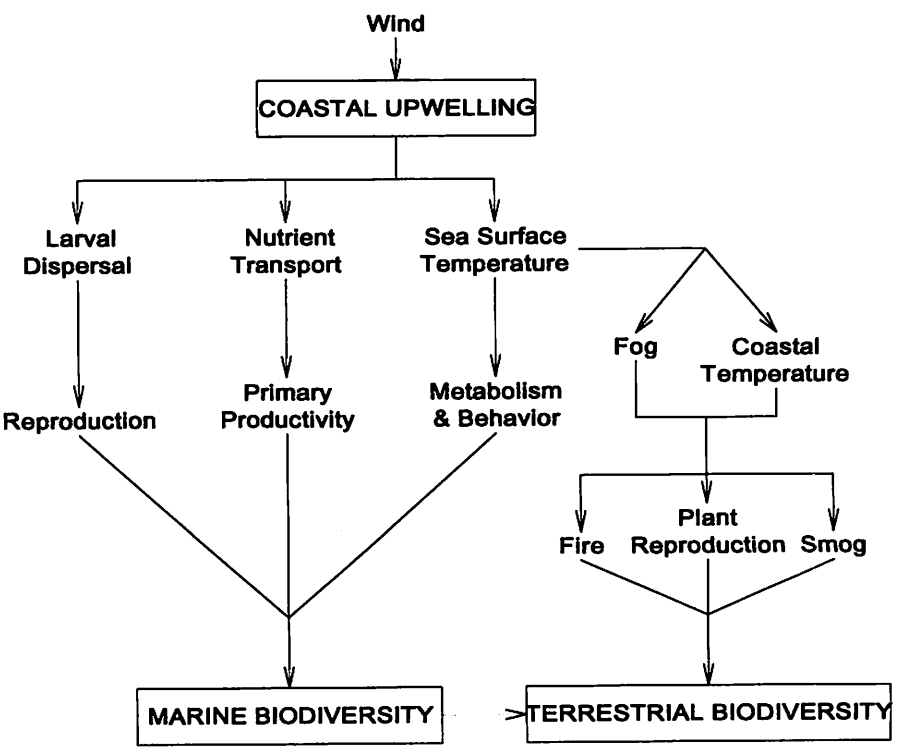

Figure 1. Some of the biological, physical, and chemical linkages between coastal upwelling and biological diversity.

grids.

A preliminary analysis suggests that the upwelling season may peak later along the coast and that annual upwelling magnitude will increase slightly in some areas and decrease slightly in others. It is worth noting that four different methods of using GCM wind output all predicted a 50% decrease in upwelling in this region in July. It is premature, however, to have confidence in predictions of possible ecological effects; improved wind data from GCMs are needed, as explained below.

Global circulation model predictions of the wind fields that cause upwelling are generally inadequate for modelling upwelling. Running the model with control (1 x CO_2) GCM output of monthly winds gave upwelling indices that were 10 to 20 times lower than the historical values determined by Bakun (1973) and Mason and Bakun (1986). One reason for these low estimates is that the GCM monthly winds are averaged as vectors.[4,5] GCM outputs of wind drag (proportional to wind squared at each time step) can be used in upwelling calculations to avoid this problem.[6] Unfortunately, the drag components may overestimate upwelling under conditions of high wind variability. In addition, we found that upwelling calculated with wind drag from the GISS control scenario had an inverted seasonal pattern south of 42°, with upwelling peaking in the winter.

A second reason for the low estimates is that surface roughness and wind speed vary significantly within the GCM coastal grids, which include both land and sea-surface topography. An improvement to the roughness averaging may await models of finer spatial resolution.[7,8]

Figure 2 shows several different mechanisms by which wind influences marine ecosystems. Note that for each process, different kinds of GCM output are needed. For example, wind drag can be used for research on upwelling and currents, but not for turbulence; monthly means are probably adequate for modeling currents, but finer time scales are needed for the other processes. Getting the most appropriate GCM output (to use as input) depends on knowing the properties of the mechanism or process being modeled.

Other methods for generating climate change scenarios for upwelling modeling include canonical correlation analysis (Graham et al., 1987a, 1987b) and using the percent change in GCM wind output to generate new wind scenarios.[9]

Historical records can also be used as a way to assess possible changes in upwelling due to global warming (U.S. EPA, 1989). Historical analogues could be generated by grouping the upwelling indices from the anomalously hot (or cold, wet, dry, or windy) years of this century. If upwelling differs among groups, it could indicate that upwelling is sensitive to certain climatic anomalies. In addition, comparing groupings for hot and cold years may give information as to how well correlated upwelling is with temperature—a useful correlation since temperature can be predicted with more confidence than can surface winds. Historical observations of marine ecosystems during interannual upwelling anomalies may provide insight into the impacts of long-term changes in upwelling.

Conclusion

Given the reliance of past research on temperature changes as proxies for climatic change, one important conclusion of this inquiry is that wind and wind-driven processes may be a component of climatic change, for natural systems and for society. Therefore, it is significant that current wind predictions are largely unsuitable for analyses of many processes, such as upwelling and wildfire (Torn and Fried, 1992; Harte et al., 1992). Specific shortcomings identified by this project include the lack of "ground-truthing" for wind output from control simulations, the gross temporal aggregation of readily available GCM output, the bias inherent in current (vector-) averaging methods, and the gross spatial aggregation of surface roughness parameters.

In general, we also suggest that temperature changes alone may not be the most important measure of climate change for predicting the response of ecological and biophysical systems to global warming. Currently, the quality of wind predictions is probably the most limiting factor in simulating the impacts of climate change for many biophysical processes, including upwelling and wildland fire in

	CURRENTS	UPWELLING	TURBULENCE
DEPENDENCE ON WIND SPEED	Square (W^2)	Square (W^2)	Cube (W^3)
DEPENDENT ON WIND DIRECTION	Yes	Only Alongshore Component	No
TIME SCALE OF VARIABILITY	Seasons	Weeks	Hours
SPATIAL SCALE	Oceanic	Coastal	Local

Figure 2. Wind-driven oceanic processes and attributes affecting choice of climate change scenario and climate change data for modeling potential responses of these processes to climatic change.

California (Harte et al., 1992; Torn and Fried 1992).

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Footnotes

[1]The spawning strategies of many Pacific Coast fish are adapted to specific drift conditions, favoring winter spawning when advection is slow or a rapid larval stage (Parrish et al., 1984). Persistent changes in advection and upwelling due to an altered climate would likely interfere with recruitment in some of these species.

[2]Global climatic change will increase fire severity in California according to model simulations of wildfire based on climate predictions from three general circulation models and 25 years of historical weather and fire observations (Torn and Fried, 1992).

[3]GCMs are super-computer models of the Earth's climate, based on systems of mathematical equations that represent the physical laws of the climate system (e.g., conservation of energy and momentum). The Earth's surface and atmosphere are represented in GCMs as three-dimensional grid cells with horizontal dimensions from 4° latitude by 5° longitude to 8° by 10°. The system of equations is solved numerically for mid-points of these relatively large grid cells. Simulations are characterized by low spatial resolution. Even so, 10 hours of CRAY-XMP processing are required to simulate one year's weather using a GCM (Mearns et al. 1990).

[4]GCMs generate vectors representing wind speed and direction at time intervals on the order of 90 minutes. Monthly mean wind speed can be computed by calculating the wind speed at each time step and averaging these scalars over the month, or it can be calculated by computing an average monthly wind vector and calculating the magnitude of this vector. As with most GCMs, the latter method is used at GISS. Shifts in wind direction over the month result in vector-derived average wind speeds that are less than averages of the scalars. For example, if the wind blew directly east half the month and west for the other half, the vector-derived monthly average wind speed would be reported as zero! In summary, vector averaging underestimates wind speed and thus underestimates upwelling relative to scalar-averaged output from the same GCM simulation. (The average direction of the wind is not affected by the method of averaging.)

[5]Other GCMs, notably the United Kingdom Meteorological Organization (UKMO; Wilson and Mitchell, 1987) and Oregon State University (OSU; Schlesinger and Zhao, 1988) compute scalar wind speed for each modeled time interval, then average these scalars to obtain monthly wind speeds. Unfortunately, these GCMs now making scalar-averaged wind speed available have large grid sizes (8° x 10°) that may render output too spatially coarse for estimating upwelling at different points

along the west coast of the United States.

[6]GISS also calculates the wind drag and the magnitude (scalar) of wind drag at each time step. To compensate for the problem of underestimating windspeed, GISS recommends calculating monthly wind speed scaling factors using the following combination of wind and wind drag output:

Scaling Factor = $W_d/W_c \times$

(monthly mean magnitude of wind drag)/

$(U_d^2 + V_d^2)^{1/2}$;

where $W = (U^2 + V^2)^{1/2}$; U and V are mean monthly east and north wind components; U_d and V_d are mean monthly east and north components of wind drag.

We analyzed two grid cells in California and found that these "scalar-averaged" windspeeds showed a greater change between simulations than did the vector-averaged wind speed.

[7]Climate modelers express relatively greater confidence in GCM predictions of high-altitude mass air flow than in GCM estimates of surface wind speeds, which are derived from high altitude winds and topographic and surface roughness parameters. A single value represents surface roughness of an entire grid cell, which in this analysis spans from the flat ocean to inland of California's coastal ranges. The resulting grid-average surface wind speed and direction are unlikely to be representative of the coastal area where upwelling occurs, although the direction of this error has not been determined. However, the *change* in wind speed between GCM warming and present climate simulations may be more robust (see footnote 9).

[8]To improve GCM-based wind predictions for predicting upwelling or other physical processes, wider availability of scalar-average wind speeds and daily or weekly averaging of 90-minute time-step output (rather than monthly averages) of climate variables would be highly desirable (Fried and Torn, 1990). Site-specificity of wind predictions would be enhanced by correlating local surface wind speed observations with high-altitude wind or pressure data. These correlations could then be applied to GCM high-altitude wind output to generate scaling factors for surface winds.

[9]For many climate variables, GCM output may be better suited to predict *changes* in meteorological patterns between the control and greenhouse climates, than to predict the pattern of either of those climates individually (U.S. EPA, 1989). For example, with temperature, every value in the historical record may be adjusted by the change (from control to 2 x CO_2 climate) suggested by the GCM output. The change may be represented by the arithmetic difference ($T_{new} = T_{historical} + (T_{2xCO_2} - T_{control})$) or by the percent change ($T_{new} = T_{historical} \times (T_{CO_2}/T_{control})$; temperature in Kelvin). There is no simple way to use this method, however, when one is concerned with both wind speed and direction simultaneously. Since upwelling is a function of the magnitude of wind stress in the offshore direction, upwelling is calculated with both these components of wind.

Structural Characterization of Fish by NMR Imaging

University of California, Davis
R/NP-1-18G
Project Initiated: March 1, 1989
Project Completed: September 30, 1989

J. Bruce German

Introduction

Fish as food represents an extremely valuable commodity. The optimal use, control, and preservation of this resource is of considerable importance not only to the producers but arguably to the nutrition of the country as well. Fish is perhaps the only flesh food in which the fat component has both a beneficial nutritional perception as well as an organoleptic one. Flesh texture is an important consideration in the quality of fresh, frozen, and harvested fish. In all cases, the quantity and distribution of fat are important but unknown values.

The quantity and distribution of fat in fish tissue varies significantly with species, age, environment, and processing. A method of rapidly describing the quantity and distribution of fat in individual specimens would be a valuable start in developing an understanding of the ways to improve both the amount and stability of fat and even to reduce defects in texture in fish flesh provided to the consumer. An important impediment to this overall research direction has been an inability to characterize the structure of fish tissue at the organizational level. The very precise chemical techniques available to measure protein, fat, and water at the level of proximate and biochemical analyses do not provide information on the structure and distribution of these macrocomponents. A technique to describe the spatial distribution of the major components in fish tissue nondestructively would be an important advance in understanding the relationship of structure to quality and possible changes in processing methods to retain or improve the final value of the product.

We have been investigating the application of nuclear magnetic resonance (NMR) imaging to the study of foods. Magnetic resonance imaging (MRI) is a new technology in which–simply put–the intensity of a signal associated with a resonant magnetic nucleus (i.e., hydrogen, ^1H), in a magnetic field can be assigned to a particular volume element in that magnetic field. One can reconstruct two- and even three-dimensional images based on the presence of a nucleus in space and several properties of its chemical environment. The strengths of MRI are that the image is thus quantitative as well as qualitative, a wide breadth of chemical information is available to this technique, and it is noninvasive. Flesh, which consists of a very heterogeneous structure of water, protein, and fat, is extremely difficult to describe if these separate components are not identified; techniques currently available require fixing or extraction and are destructive, but MRI can be performed even on living material.

Our objective in this proposed project was to use MRI to describe the distribution of water and fat in whole, intact fish. To accomplish this goal, we first investigated pure oil and water samples and oil in water emulsions to develop spin-echo protocols for relaxation-timing parameters for water-weighted and oil-weighted images. These relaxation-weighted protocols were then translated directly to imaging intact trout. As this technique develops, researchers will be able to use the methodology in structural studies of the effects of such variables as types of species, harvesting, processing, and freezing.

Methodology

A variety of edible oils were used to establish NMR parameters for

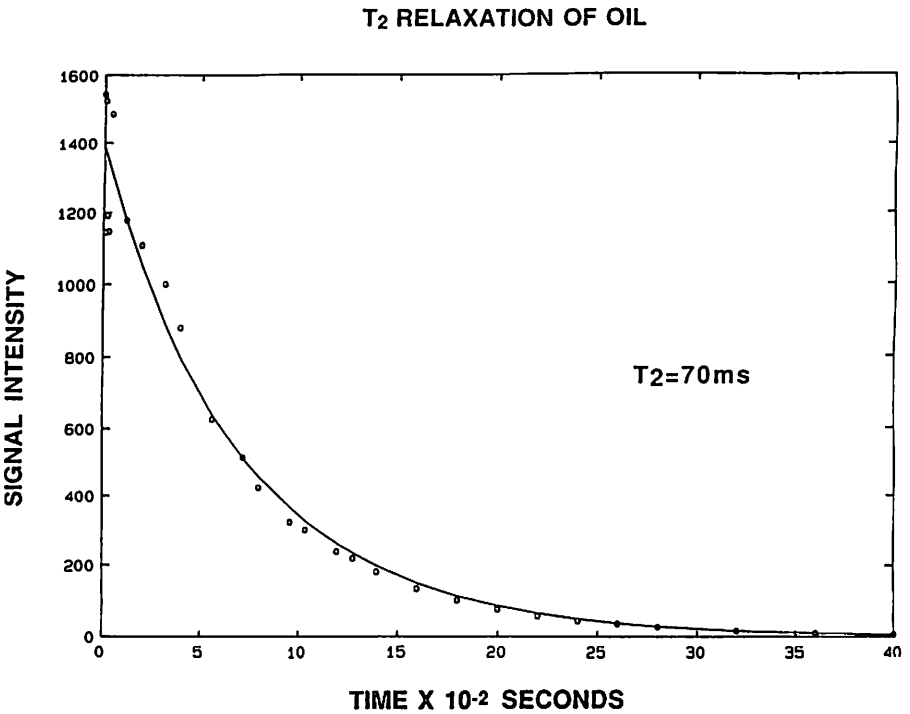


Figure 1. T_2 relaxation time determination for oil. Signal intensity as a function of time is plotted, and the fitted curve for a single exponential is superimposed on the data.

flesh studies, including tricaprin, triolein, trilaurin, and mixed unsaturated triglycerides obtained from Capitol City Products. Freshwater rainbow trout (approximately 2 kg) were obtained from the aquaculture facilities at the University of California, Davis.

NMR measurements of the oil-water systems at 24°C were obtained using a General Electric CSI-II, 2-Tesla, multinuclear imaging spectrometer tuned to 85.5 MHz. A 0.15-m (internal diameter) imaging coil (General Electric Medical Systems, Fremont, California) was tuned to measure the ^1H signal.

Spin-lattice relaxation values were determined using the inversion-recovery method (180°-t-90°) (Abragam, 1961). Spin-spin relaxation values were determined using the Hahn spin-echo method (90°-TE/2-180°-TE/2) (Abragam, 1961).

Magnetic resonance spectra of the oil-water systems were recorded using 256-point resolution and a 156-mm field of view. Spectra were obtained with two signal acquisitions. Enhancement of the ^1H signal from the oil component was obtained using a T_1 weighted, spin-warp, spin-echo image sequence with a predelay (PD) time of 220 ms and an echo time (TE) of 12 ms. Enhancement of the ^1H signal of the water component was obtained using a T_2 weighted imaging sequence with a PD of 1,800 ms and a TE of 225 ms. Experimental parameters for fish samples included four signal acquisitions, 256-point resolution, and a field of view of 102.3 mm. The pulse sequence for enhancement of the water in fish samples included a PD of 1.0–2.0 s and an echo time of 80 ms. For enhancement of the lipid component, the pulse sequence included a PD of 200 ms for all samples and echo times of 10–15 ms.

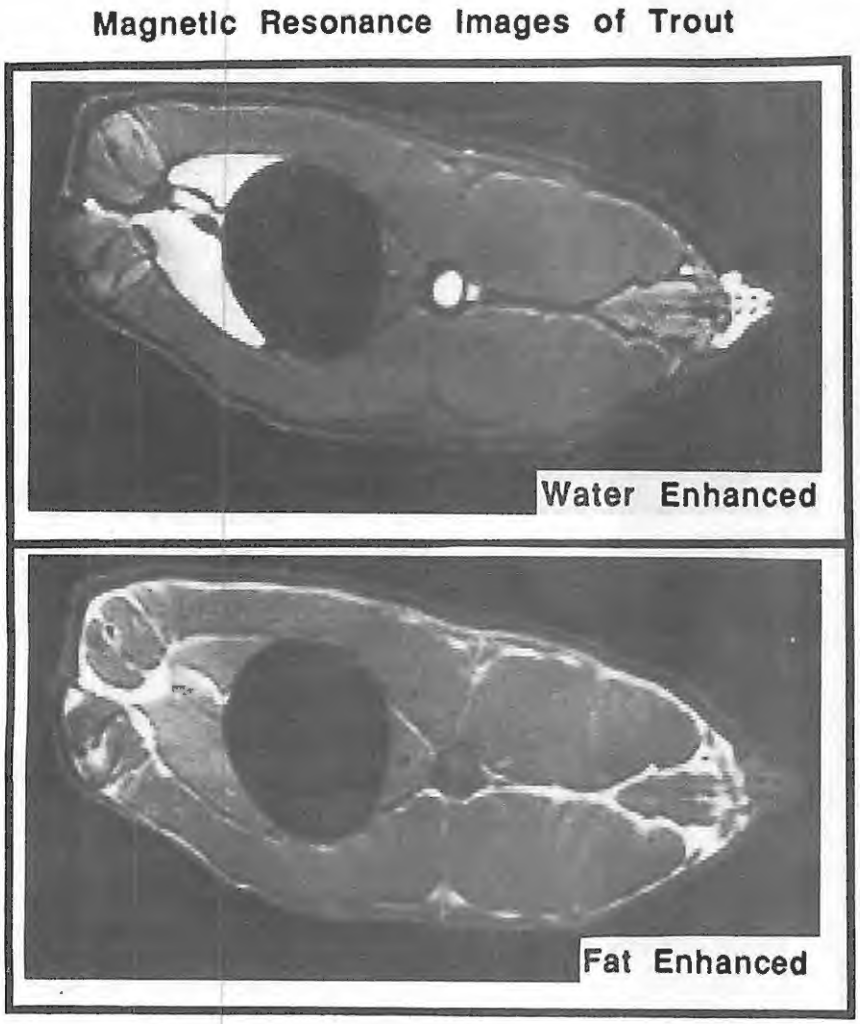


Figure 2. Magnetic resonance images of intact trout. Two-dimensional, vertical planar slices approximately 2-mm thick were assembled, and images were reconstructed. A gray scale from dark to light corresponding to increasing signal intensity was used to project the images. Relaxation-weighted protocols were used in the imaging; thus, in the water-enhanced image, increasing lightness represents increasing density of water. In the fat-enhanced image, increasing lightness corresponds to increasing density of fat.

To investigate the effect of freezing fish, three experimental treatments of the fish before imaging were implemented, including storing fish at –70°C for 8 hours or at 4°C for 8 hours, and holding fresh fish at ambient temperature for 1 hour. After a fresh fish was measured, it was then frozen to –70°C and measured again. All fish were removed from storage conditions 12 hours before experimental measurements were taken.

Spin-lattice relaxation times, T_1, were determined for the fresh, refrigerated, and frozen fish. Basic inversion-recovery sequences have been found not suitable for large, inhomogeneous samples such as these since the total signal present could not be accurately measured. Therefore, a pulse sequence referred to as SUFIR (super fast T_1 determination by inversion recovery), which allows approximation of the T_1 based on only a slice of a sample rather than the whole sample, was used to measure spin-lattice relaxation times (B. Kauten and M. McCarthy 1989, unpublished results). Spin-spin relaxation times, T_2, were determined for each condition studied. T_2 values were determined from a curve fit of the signal intensity obtained using different TEs in the MRI pulse sequence.

The 0.10-m imaging coil was used to obtain 256-point by 256-point (256 x 256) magnetic resonance images of the fresh trout that was measured, frozen, and measured again. The 0.15-m imaging coil was used to obtain 256 x 256 images of the

refrigerated and frozen trout; this coil was also used to determine the spin-lattice and spin-spin relaxation times.

Results and Discussion

The information content of the MRI signal from a volume element within a sample can be controlled by properly selecting the pulse-sequence parameters. A magnetic resonance image results from measurement and reconstruction of the signal intensity of volume elements throughout a sample. The NMR signal from each volume element resulting from a standard spin-warp, spin-echo pulse sequence can be approximated by the following phrase (Morris, 1986):

$S@r(1-\exp(-PD/T_1))\exp(-TE/T_2)$ [3]

where S is the signal intensity, and r is the density of the nuclei. The predelay (PD) and the echo time (TE) are variables set for each type of experiment. The signal intensity represents information on the density and relaxation behavior of the nuclei. A long PD (in comparison to spin-lattice relaxation) and a short TE (in comparison to spin-spin relaxation) are required for accurate representation of the entire nuclei density by the recorded signal.

In a system in which hydrogen nuclei from two different components, such as oil and water, contribute to the total signal, one is still able to differentiate between the components. To distinguish oil from water, one needs to select for the individual relaxation behaviors. Magnetic resonance spectra can be generated such that signal intensity at any point is a function of the hydrogen density weighted by the relaxation values. This method allows one to suppress the nuclei signal from oil separately from that of water.

In the first series of experiments, spin-lattice and spin-spin relaxation values were determined for individual oil and water samples. Figure 1 illustrates the spin-spin relaxation, T_2, of triolein oil. Signal intensity is plotted against the time during which the signal decays. The relaxation function fitted acceptably to a single exponential decay, indicating that the oil can be characterized by a single time constant. Spin-lattice relaxation, T_1, of triolein was also characterized by a single time constant. Similar relaxation analyses completed on water also characterized it by single time constants. Results of these experiments show that triolein, for which T_1=220 ms and T_2=70 ms, exhibits significantly different relaxation behavior than water, for which T_1=1,800 ms and T_2=720 ms. The validity of this approach on more complex models was verified using an oil-in-water emulsion (data not shown).

These protocols were then used to image fish. Figure 2 (top) illustrates the resolution of fourier-imaging, spin-echo experiments using intact freshwater trout. This experiment was essentially weighted to aqueous hydrogens and hence provides a spatial resolution of the water content of the tissues. Several features of the flesh and internal organs are visible owing to their inherent differences in water content.

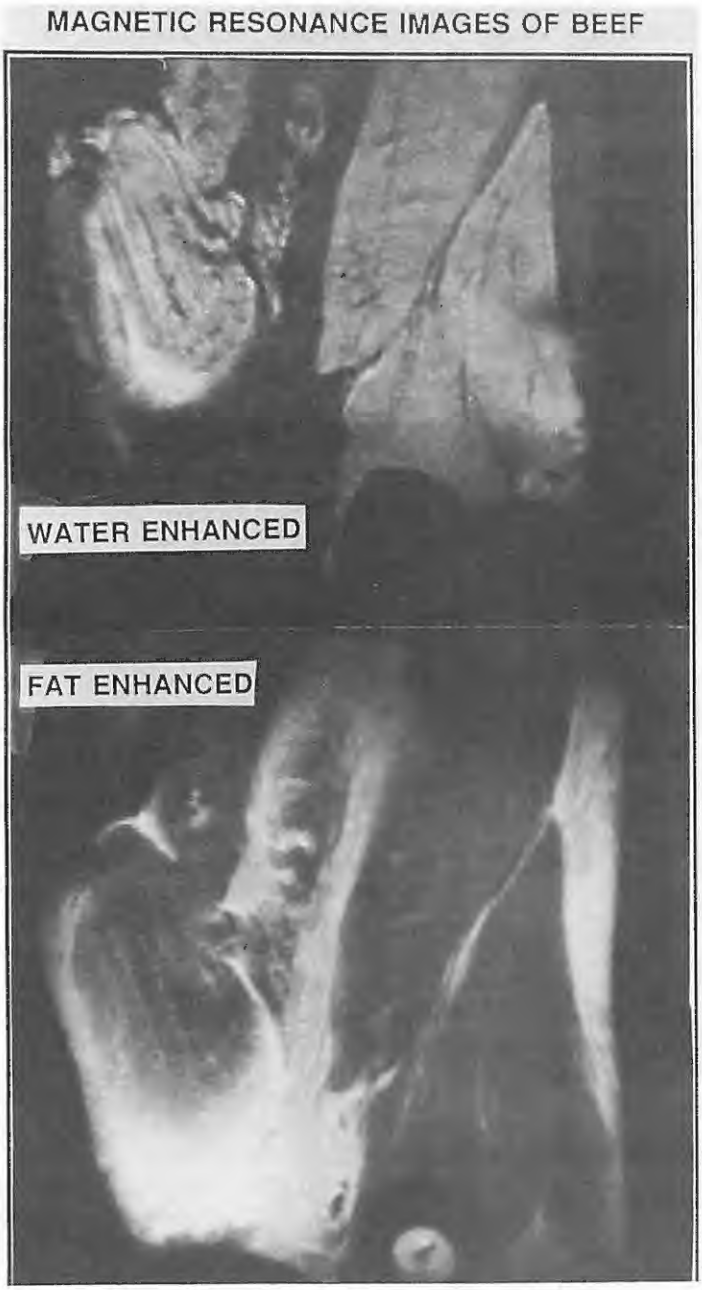


Figure 3. Relaxation-weighted images of red meat: water enhanced and fat enhanced. Protocols as described for Figure 2.

Figure 2 (bottom) summarizes the results of spin-echo experiments designed to image the same fish on the basis of the fat, separately using its different relaxation properties. The results confirmed proximate analyses that trout, a lean-muscled animal, accumulates fat subcutaneously, primarily in the belly flap region, and does not deposit large quantities of fat intramuscularly. Contrast this with images of red meat (Figure 3), in which fat is deposited in large quantities both subcutaneously and intramuscularly.

Developing this analytical technique for describing the distribution of fat in intact fish was our major objective in this project, and we feel that this has the potential to be very useful in future studies of a variety of physiological and quality properties of fish. We stress that these analyses are entirely noninvasive and can be performed even on live tissue if the tissue is immobilized. The technique used on female trout samples readily distinguished developing eggs and showed significant variability in the population of eggs within the female (Figure 4).

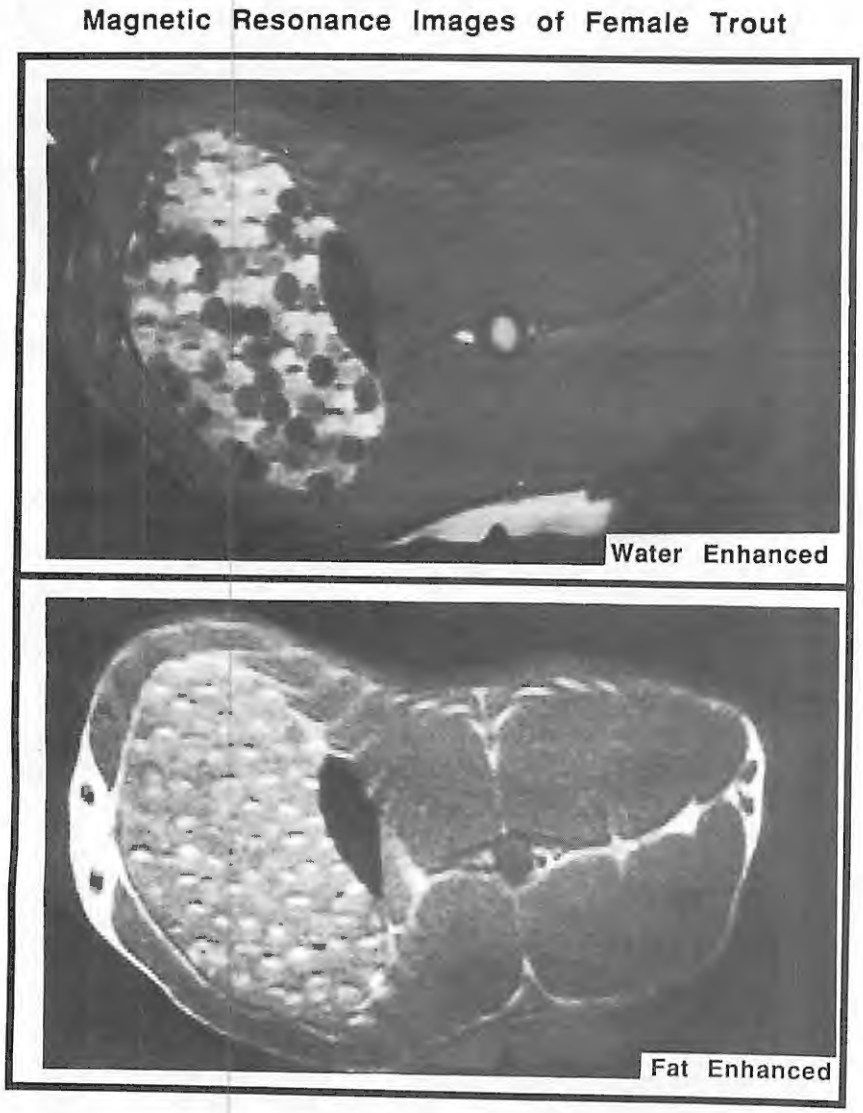


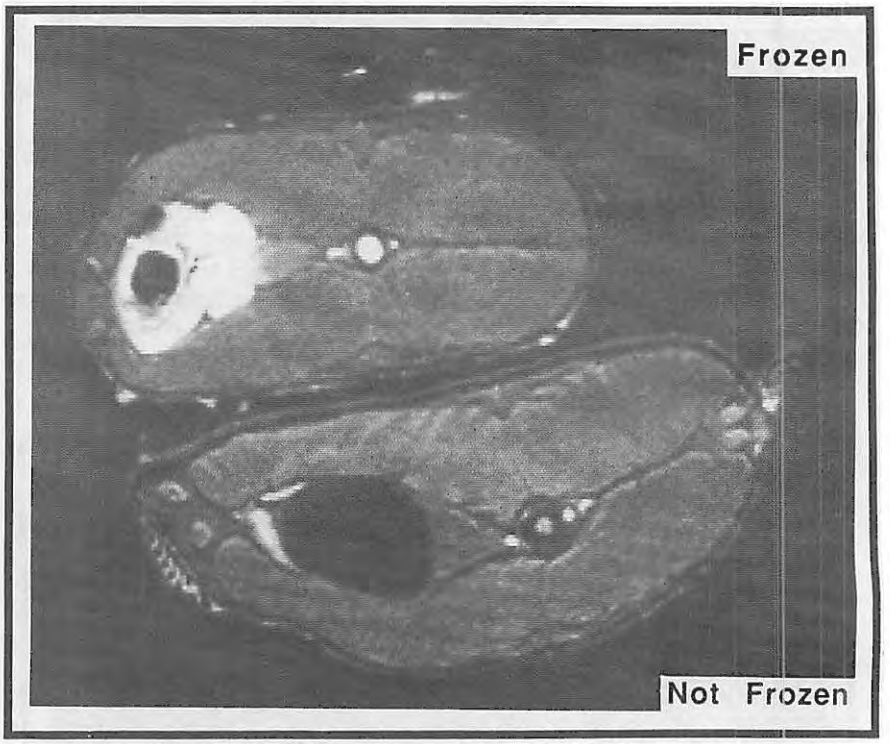
Figure 4. Relaxation-weighted images of mature female trout containing eggs: water enhanced and fat enhanced. Image reconstruction protocols as described for Figure 2.

The effects of freezing were studied with this new technique. Previous studies on alterations in water behavior in frozen tissues suggested that the relaxation-weighted imaging protocols that we had developed might help discern the effects of freezing. The novel SUFIR techniques used to obtain averaged relaxation times for intact fish require that data be fitted to single exponentials. There appeared to be essentially no statistically significant difference in the spin-lattice relaxation times or the spin-spin relaxation times among the experimental treatments. The T_1 and T_2 values for the fresh, previously refrigerated, and previously frozen intact fish were similar. This may largely reflect the inability of averaged relaxation times to distinguish the subtle structural alterations caused by freezing. The relaxation behavior of intact fish would not be expected to follow a single exponential relaxation behavior overall, and hence our technique of forcing this may have lost information necessary to distinguish the effects of freezing. Images of the fresh and previously frozen fish are shown in Figure 5 (water enhanced and fat enhanced). Differences between the fresh and previously frozen fish were not apparent.

Thus, although the technique will readily distinguish certain structural alterations, differences in the inherent relaxation behavior of the tissues due to freezing and thawing were too subtle to be readily distinguished in the immediate imaging experiments. Therefore, we must conclude that characterization of processing effects such as freezing, which generate subtle alterations in physical properties of the material, will require further research to develop imaging protocols that can demonstrate these differences.

In summary, spin-echo imaging

Magnetic Resonance Images of Trout: Water Enhanced



Magnetic Resonance Images of Trout: Fat Enhanced

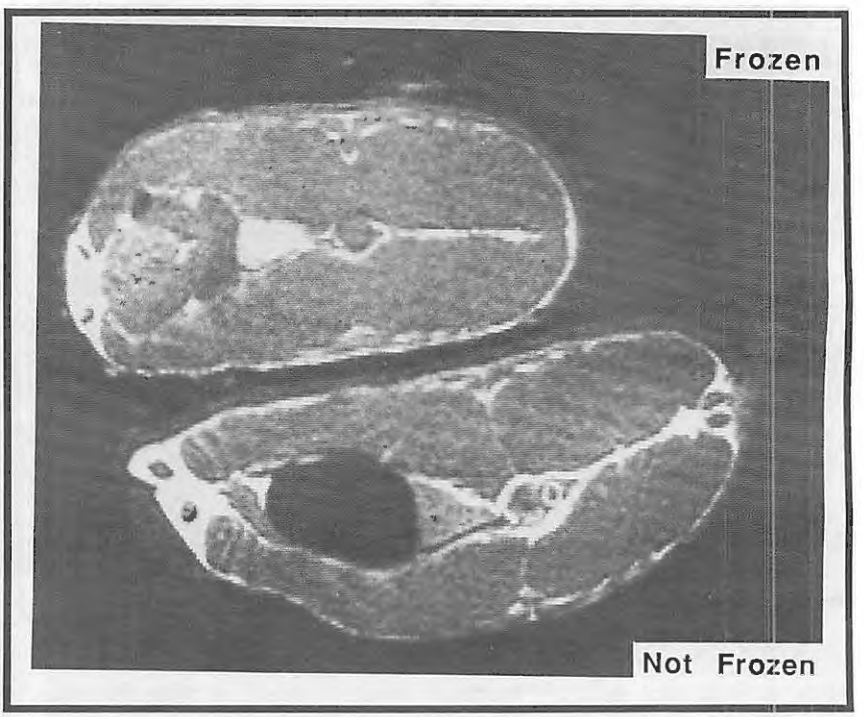


Figure 5. Relaxation-weighted images of intact frozen or fresh trout, images water-enhanced and fat-enhanced.

techniques were developed that readily distinguish the structure of fish meat and the distribution and abundance of fat. These relaxation-weighted approaches to imaging appear to be useful for determining a variety of structural features of fish as food. Further research is necessary to further develop the techniques to detect subtle effects of processing and storage abuse.

Cooperating Organizations

Aquaculture Research Project, University of California, Davis
California Dairy Center
Capitol City Products

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Genetic Divergence Between Reproductive Types in Northern and Southern Populations of the Edible Goose Barnacle, *Pollicipes*.

University of California, San Diego
R/NP-1-18M
Project Initiated: October 1, 1989
Project Completed: September 30, 1990

Robert J. Van Syoc and William A. Newman

Introduction

The edible intertidal goose barnacle, *Pollicipes*, is represented by three hermaphroditic species having planktotrophic larvae: *P. pollicipes* in the northeastern Atlantic, *P. elegans* in the tropical eastern Pacific, and *P. polymerus* in the northeastern Pacific (Newman and Killingley, 1985).

Pollicipes pollicipes, used for centuries as food in Portugal and Spain, ranges from the Bay of Biscay to North Africa, but commercially available stocks have largely been depleted in recent years, and both species from the eastern Pacific are now being imported (anonymous, 1987).

The more or less tropical species, *P. elegans*, ranges from southern Baja California to Peru. It is commercially abundant at the southern end of its range, in northern Peru (Kameya and Zeballos, 1988). In Lima, it is available in fish markets (personal observation), served in restaurants, and exported to Spain.

The third species, *P. polymerus*, abundant pretty much throughout its range from southern Alaska to southern Baja California (Newman and Abbott, 1980), is not currently harvested commercially except in British Columbia, and then for export to Spain (anonymous, 1987). Gibbons (1964), who notes that the California State Fish and Game Commission recommended *P. polymerus* as food and published a recipe for it in 1916, discusses methods for collection and preparation. Despite this notice of commercial value, this northeast Pacific resource remains largely unexploited.

The general distribution and biology of *P. polymerus*, reviewed by Newman (1979) and Newman and Abbott (1980), are relatively well known. It ranges from Alaska south, through the Oregonian Faunal Province, across the Californian Transition Zone, and into the Californian Faunal Province all the way to the southern end of Baja California, where it is sympatric with the northern population of *P. elegans*. It has been generally considered that populations of *P. polymerus* north of Pt. Conception grow more slowly and breed seasonally, whereas those to the south not only grow faster, but are reproductively active year-round (Cimberg, 1981). However, until recently, no genetic differences between these populations had been suggested.

Reproductive Patterns in *Pollicipes polymerus*

In field and laboratory studies of reproductive activity in *P. polymerus*, as indicated by the percentage of individual barnacles brooding, Cimberg (1981) found two major reproductive patterns: a northern one, in which maximum brooding activity is achieved when the surface temperature of the sea is rising to around 14°C; and a southern one, which also requires rising temperatures, but to 20°C. He attributed this difference to the sharply separated marine climates experienced by the northern and southern populations, on either side of the so-called Californian Transition Zone, which is centered on Pt. Conception at 35°N (Newman, 1979).

Cimberg (1981) also noted the presence of a third reproductive type south of Pt. Conception; namely, populations in which the percentage of barnacles brooding increases as temperatures fall toward 14°C. From these findings, he infers that (1) members of this population represent waifs of the northern type that have been transported and established south of Pt. Conception and (2) as the waifs can occur in the same geographical region as the southern type, the difference in their reproductive pattern must be genetically rather than ecotypically determined.

Populations Genetics

The apparent genetic difference between these stocks needs to be identified and quantified. Although enzyme electrophoresis studies could possibly detect some differences between the northern and southern groups, DNA sequencing can give us a more accurate idea of the exact amount of any genetic difference within and between them. In addition, these sequences can be recorded and compared directly with those of other subpopulations of the species or even different species.

Genetic variation has been described in contiguous western Atlantic populations of the horseshoe crab, *Limulus polyphemus* (Saunders et al., 1986; mitochondrial DNA restriction fragment length polymorphism). Therefore, there is more than an intuitive basis for expecting that subpopulations of *P. polymerus* might show genetic divergence despite apparent opportunities for significant gene flow via planktonic larvae and cross-fertilizing hermaphroditism. Although the differences in growth rate and size at maturity between onshore and offshore populations of *P. polymerus* are likely due simply to the differences in environmental conditions (Page, 1986), it is important to determine if a genetic component can be completely ruled out. Direct information on how much genetic variation exists between such more or less contiguous populations, as well as along the latitudinal environmental gradient disrupted by the Californian Transition Zone, is highly desirable.

Thus, the focus on this traineeship

has been the acquisition of skills and techniques needed to extract and sequence mitochondrial DNA (mtDNA) in order to determine if northern and southern populations of *P. polymerus* are genetically distinct.

Methods and Materials

Pure DNA was prepared for sequencing, after extraction and separation from muscle tissue of *P. polymerus*, by the polymerase chain reaction (PCR; Mullis and Faloona, 1987). PCR was essential to the project, because we have discovered that the level of mtDNA is relatively low in this barnacle, as compared with crab (*Loxorhynchus grandis*) tissue.

A protein coding gene, the cytochrome oxidase subunit 1 (CO1) gene of the mitochondrial genome, was selected for study because it allows us to determine the synonymous base differences between individual barnacles. Synonymous changes do not result in amino acid replacement and are therefore less subject to external environmental influences and natural selection (DeSalle et al., 1987).

Our successful amplification of the CO1 gene by using PCR has enabled us to prepare DNA from this gene from many individual barnacles to study genetic variation within and between populations of *P. polymerus*.

Results and Discussion

We have used the DNA acquired through PCR to obtain some sequence data for *P. polymerus*. However, some technical problems remain in the sequencing of this gene. Our own experience, and that of colleagues doing similar work on other organisms, has led us to think that additional molecular tools must be developed before we can obtain long sequences routinely.

To address this problem, we have sequenced specific portions of the target DNA. These have been compared with DNA sequences from this gene known from other organisms and are being used to design new primers (oligonucleotides) for PCR and sequencing. This will be especially useful because of the small amount of mtDNA in barnacle tissue and the problems associated with direct sequencing of PCR products.

Although it is difficult to sequence directly PCR products from organisms that are relatively unknown from the standpoint of molecular biology, progress is being made. The project continues with the knowledge that nucleotide sequencing of homologous DNA fragments offers a larger data set for statistical manipulation than restriction-site maps or patterns on electrophoretic gels. Also, recorded sequence data can be compared directly with newly acquired data from other individuals in the population, with other populations, and even with other species for a better understanding of breeding patterns and evolution in marine organisms.

Such information on the genetics of regional stocks and the likely source of larvae will be important when management decisions must be made.

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California Red Sea Urchin Fishery

University of California, Davis
R/NP-1-18N
Project Initiated: October 1, 1989
Project Completed: September 30, 1990

Louis W. Botsford

This rapid response project provided funds, in anticipation of a Sea Grant project that began in October 1990, for one trainee to continue development of an experimental approach for harvesting and monitoring recruitment of red sea urchins. Laboratory work was done by the trainee, Laura Rogers-Bennett, with advice and direction from meetings with the principal investigators, L. W. Botsford, James Quinn, and Chris Dewees.

Experimental Harvest

When this project originated, the experimental harvest was conducted in 8-m x 8-m treatment sites, with three treatments (lower size limit, lower and upper size limits, and control) at each of two depths (5 m and l0 m). Because rampant migration into the treatment sites was observed, the first task under current funding was to try harvesting in naturally isolated urchin beds. We extended the two harvest treatments to complete beds at the 5-m depth, and migration was observed again.

To isolate treatments, we next tried several methods for preventing migration that had been used earlier by intertidal researchers. We tested copper flashing by placing a 0.3-m strip across the bottom and sides of a 3-m x 6-m tank. In 7 days, 5 of 40 urchins in the tank had crossed the barrier, and 15 of the 40 were dead. We did not consider copper flashing further. We tested tanglefoot (Tanglewood Company, Grand Rapids, Michigan) by spreading it in a 0.15-m swath across the bottom and sides of a 20-gal (85 l) aquarium, with urchins on one side and kelp on the other. In 20 min, the urchins had negotiated the tanglefoot.

We tested 2.5-cm mesh net fences, 0.5 m high with lead core line on the bottom and floats on the top by stretching it across the bottom and up the sides of a 4 m circular tank. None of the 10 red urchins on one side crossed to the other side even when kelp was placed there.

We then decided to try the nets in the subtidal areas. To increase replication of our experiments, we divided the original 8-m x 8-m plots into four 4-m x 4-m plots, one for each treatment, one for an unharvested control, and one for an unfenced control. We plan to try three of these at each of two depths. We deployed one of these setups at a 5-m site with chain as the lead line. Within several weeks, after a summer storm, the nets came loose because the fasteners had pulled out of the rock. We then tried small bolts with anchors in setting up a site at l0 m. That was deployed in October 1990, so we have no results yet.

We also tried chain without nets, but coated with antifouling paint, in subtidal areas. This did not work.

Larval Collectors

In late May 1990, we deployed experimental larval collectors that consisted of scrub brushes lashed back to back and attached to a line about 1 m from the bottom, held up by floats. These worked well, though we caught crabs and other invertebrates, rather than urchins. We attempted to remove organisms by using a sonicator, but it did not work very well.

In our current scheme, each collector has two sets of brushes, one near the bottom and one at about 1 m just below two plastic floats. We have deployed three transects of these at Bodega Marine Laboratory, placed at depth intervals of about 2 m. The three transects vary in how protected they are. The first collections from these yielded two settled urchins. Our protocol includes removal of the organisms by immersion in magnesium chloride; quantification of potential predators; occasionally, analysis of the contents of the guts of predators; and complete preservation of all organisms on the brush. We also plan to continue testing substrates besides brushes (e.g., Astro turf, Tuffy pads, algae-covered slate).

Site Survey

We surveyed the coast near Bodega Bay to find suitable locations for both additional experimental harvests and additional recruitment transects. The only other site nearby that is protected from commercial urchin harvesters is the urchin reserve outside Gerstle Cove at Salt Point. This site was surveyed and was deemed suitable for an experimental harvest.

We surveyed eight sites for suitability for recruitment transects. Features observed were substrate composition, algal cover, invertebrate density, red sea urchin density, and accessibility. Two were acceptable, in addition to Bodega Marine Laboratory, Salt Point, and Fort Ross. We plan to install three transects at each site, across an exposure gradient.

Tagging

To enable us to monitor migration and estimate growth rate, we evaluated several methods of marking individual sea urchins. Neither vital stains nor fluorescent pigment beads were satisfactory because of lack of color contrast. Soda straws attached with epoxy were rubbed off by the urchins. Floy tags used on fish led to high mortalitiy. Surgical tubing stays on for a couple of weeks before the spine is shed, and can be written on. We have put personal internal transponder tags in 12 urchins, which seems to be working, but the transponders can be read only out of water. We have not tried internal coded metal tags, which would require sacrificing an urchin to identify it. We plan to continue this effort.

Cooperating Organizations

California Department of Fish and Game, Fort Bragg

Measurement by NMR Spectroscopy of Sublethal Toxic Effects in Marine Organisms

University of Califomia, Santa Cruz
R/NP-1-19A
Project Initiated: January 1, 1990
Project Completed: September 30, 1990

Ronald S. Tjeerdema

Introduction

Description of the sublethal effects of toxicants on aquatic organisms most commonly involves tests in which morphological or behavioral responses are measured *in vivo*. Although useful for estimating harmful concentrations of chemicals, they are usually not sensitive enough to detect sublethal biochemical effects. In contrast, description of the biochemical actions of toxicants has relied on *in vitro* techniques, in which neither the responses of whole organisms nor the interactions of natural stress factors are measured. Thus, methods for measuring biochemical perturbations in live, intact animals under simulated environmental conditions are needed. *In vivo* nuclear magnetic resonance (NMR) spectroscopy meets these requirements.

The ^{31}P (phosphorus-31) nucleus is highly useful for *in vivo* NMR spectroscopy because of its high natural abundance (100%), its presence in important endogenous "energy" compounds, and the use of inorganic monophosphate (P_i) as a marker of intracellular pH (pH_i) (Gadian, 1982). *In vivo* changes simultaneously measurable in intact organisms include fluctuations in concentrations of phosphagens such as phosphoarginine (PA) or phosphocreatine (PC), nucleoside phosphates (NPs), phosphoesters (i.e., glucose 6-phosphate), P_i, and pH_i (Gadian, 1982). The proved usefulness of these measurements in the biomedical sciences should also apply to environmental toxicology.

Several reports have described the use of *in vivo* ^{31}P NMR spectroscopy with excised tissues, including the adductor muscles from cockles (Barrow et al., 1980) and mussels (Ellington, 1983), barnacle depressor muscles (Dubyak and Scarpa, 1983), abalone mantle (Burt et al., 1976), deshelled snails (Thompson and Lee, 1985), and decapsulated *Artemia* cysts (Drinkwater and Crowe, 1987). All used conventional probes that both accommodate and average over entire samples smaller than the probe. They are useful for organ localization in studies of whole organisms only if the organ can be isolated for insertion into the probe; this has been reported for crab leg muscles (Briggs et al., 1985) and the tail muscles of crayfish (Butler et al., 1985), shrimp (Kamp and Juretschke, 1987), and prawns (Thebault et al., 1987). In contrast, larger bore instruments can accommodate surface probes for localized spectroscopy, allowing signal acquisition from less discrete organs, as well as from tissues larger than the probe (Gadian, 1982). Surface probes have been used to localize tissues in intact organisms that are not discrete to conventional probes, including abalone foot (Higashi et al., 1988) and fish lateral muscles (van den Thillart, et al., 1989). However, *in vivo* NMR spectroscopy of any type has not been used previously to investigate the toxic effects of chemicals in intact aquatic animals.

The general biocide pentachlorophenol (PCP), an uncoupler of mitochondrial oxidative phosphorylation (Corbett et al., 1984), may inhibit ATP synthesis by destroying the electrochemical potential produced across the inner mitochondrial membrane by electron transport (Mitchell, 1961, 1966; Mitchell and Moyle, 1967). PCP is introduced into coastal waters from treated wood pilings, jetties, antifouling paints, and drilling muds (Rao et al., 1979). It has been widely detected in aquatic animal tissues (Pierce and Victor, 1978), and high concentrations in water have been associated with major fish die-offs (Pierce et al., 1977). At sublethal levels, PCP retards larval development in most teleosts (Dalela et al., 1980), and in grass shrimp (*Palaemontes pugio*), it retards limb regeneration and increases mortality during ecdysis (Rao et al., 1979). In both grass shrimp and blue crabs (*Callinectes sapidus*), it decreases tissue consumption of oxygen (Rao et al. 1979); in pond snails (*Lymnaea stagnalis*), oxygen consumption and mitochondrial phosphorylation are suppressed (Weinbach, 1956).

Project Objectives

The red abalone (*Haliotis rufescens*), a marine gastropod of both recreational and commercial importance, resides in intertidal and subtidal zones along the California coast. The sublethal effects of PCP in marine gastropods, including effects at the molecular level, are unknown. Because of their sedentary nature and large foot muscle, intact abalones are ideal for surface-probe-localized NMR spectroscopy. Therefore, the primary objectives of this investigation were (1) to develop an inert, flow-through NMR spectroscopic system for the dosing and biochemical monitoring of intact aquatic invertebrates; (2) to determine the sublethal effects of PCP on phosphorylated metabolites and pH_i in red abalone foot muscle by using ^{31}P surface-probe-localized NMR spectroscopy; and (3) to determine the interactive effects of emergence on PCP toxicity in red abalones.

Methods

Exposure system. A diagram of the exposure chamber is shown in Figure 1. It was an 11.7 (w) x 14.3 (l) x 6.2 (ht) cm box of clear acrylic polymer 4.5 mm thick, except for the bottom surface (adjacent to the NMR surface probe), which was 1.5 mm thick. The top was sealed with a clear acrylic compression plate (15.9 x 18.4 cm), also 4.5 mm thick, a neoprene gasket, nylon screws, and wing nuts. Abalones were placed into a 2-mm Tedlar fluoropolymer

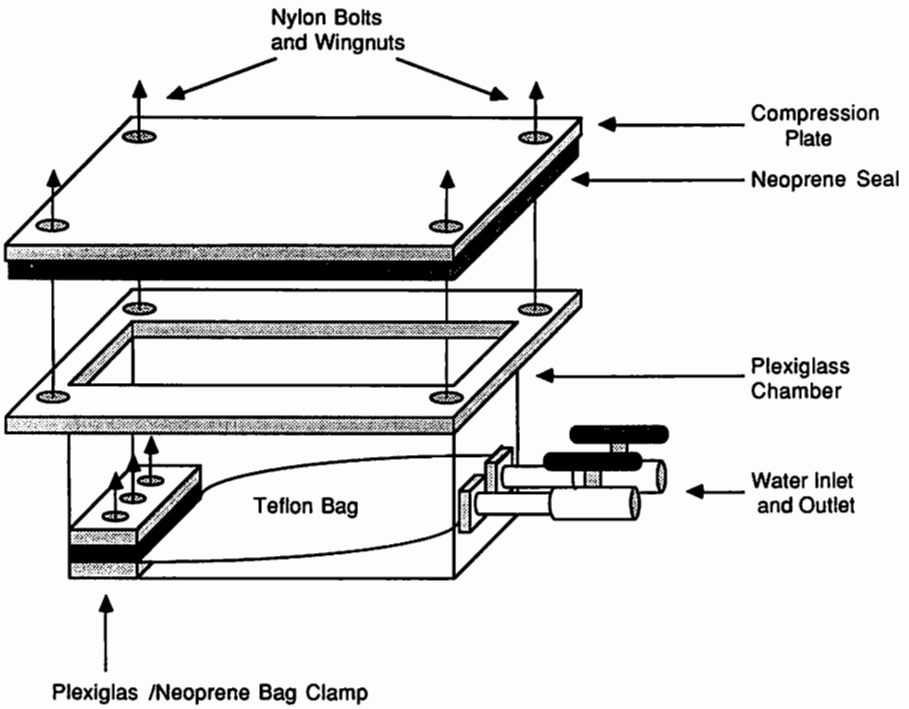

Figure 1. The fluoropolymer and acrylic exposure chamber for aquatic invertebrates. Water flow is restricted to the interior of the bag only, and the acrylic box serves as a solid support as well as a secondary water enclosure to protect the nuclear magnetic resonance magnet from leakage.

bag (12 x 15 cm; AeroVironment, Monrovia, California) equipped with two Teflon bulkhead compression tubing connectors (0.25 in. [0.64 cm] outer diameter; Fluoroware, Chaska, Minnesota) that were anchored to one end of the box; the opposite end (open for insertion of organisms) was sealed with an acrylic clamp (2.2 x 10.5 cm) consisting of a neoprene gasket, nylon screws, and wing nuts. The bag provided an inert primary containment for exposure of organisms, and the box provided both a solid support for the bag and a secondary containment to ensure against leakage.

Seawater was stored in a 20-l Nalgene (Sybron, Rochester, New York) polypropylene carboy equipped with a spigot. Temperature control was provided by a 25-ft (7.6 m) coil of fluoropolymer-coated copper heat-exchanger tubing (0.25 in. outer diameter; Ace Glass, Vineland, New Jersey) connected to a recirculating water bath, and an aquarium air pump provided aeration. Water was pumped from the carboy with a Masterflex Model 7523-00 peristaltic pump equipped with a Quick-Load pump head and silicone tubing (size 16; Cole-Parmer, Chicago, Illinois); all tube fittings were Teflon compression-type (0.25 in. outer diameter; Fluoroware). PCP stock solution was pumped into the water flow, by using a second identical pump, from a 6-l fluoropolymer gas-sampling bag (4 mil) that collapsed during emptying to eliminate headspace. The tubing leading to the chamber served to mix the flows and, for temperature control, the bag was placed in a water bath.

Toxicity range finding. To determine a sublethal concentration of PCP for use with NMR spectroscopy, we conducted 6-hr range-finding tests. Six hours was chosen to accommodate a reasonable period for NMR spectroscopy. Both broad- and narrow-range tests were performed to determine the approximate 6-h LC_{50} and no-observable-effect level (NOEL). In both tests, abalones (averaging 18 months old, 5 g, and 2.5-cm shell length) were exposed to PCP at 14°C in 125-ml Erlenmeyer flasks, and the solution (120 ml) was renewed hourly. The broad-range test involved individual abalones at PCP levels of 0, 0.5, 1, 2, 4, 8, and 16 mg/l; the narrow-range test involved six abalones per flask at levels of 0, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/L. In both tests, PCP was dissolved in 0.5 ml of methanol before seawater spiking, and flasks were slowly aerated with pasteur pipets and an aquarium air pump. After a 6-hr exposure, the flasks were refilled with clean seawater, and the abalones were checked for recovery during an additional 18 hr. Death was determined by lack of surface adhesion and movement after the full 24-hr.

PCP disposition. The same exposure system was used to measure [^{14}C]PCP disposition in foot muscle, except that a column (20 x 1.4 cm inner diameter) of Amberlite XAD-4 resin (Rohm and Haas, Philadelphia, Pennsylvania) was added onto the outlet to collect depurated residues before water discharge. The resin, which contains impurities from manufacture, was cleaned before use by sequential 24-hr solvent extractions with methylene chloride and then methanol in a Soxhlet extractor. Clean resin was stored under methanol in an amber glass bottle until use.

In order to determine the PCP concentration in abalone foot muscle after both 5-hr exposure and 13-hr depuration, six individual abalone (averaging 3 years, 89 g, and 9 cm) were exposed separately to 1.2 mg/l of PCP in seawater at 14°C. The dosing solution was prepared by adding 15 mg unlabeled PCP (sodium salt) and 0.5 mg of [^{14}C]PCP in 0.5 ml methanol to 6 l of seawater in a fluoropolymer bag to create a PCP concentration of 2.4 mg/l. The final exposure concentration (1.2 mg/l) was generated by combining flows of 20 ml/min from both the carboy and the PCP stock bag to produce a total flow of 40 ml/min through the chamber.

After exposure, three abalones were immediately removed from the chamber to measure bioaccumulation; while the others were exposed to clean seawater for

an additional 13 hr to allow depuration; all six were sealed in polyethylene bags and immediately frozen. The resin columns were replaced after both uptake and depuration. Finally, parallel controls were run, with methanol only, to determine possible toxic effects and to check for unlikely intrinsic radioactive residues in foot muscle and without abalones to determine ^{14}C recovery from the system.

Foot muscle (0.2 g) was homogenized with a Biohomogenizer tissue homogenizer (Biospec Products, Bartlesville, Oklahoma), solubilized by incubation (50°C, 18 hr) in 2 ml of tissue solubilizer, and quantified for ^{14}C by liquid scintillation counting (LSC) with a Packard Tri-Carb Model PL scintillation spectrometer; samples were corrected for background, quenching, and efficiency. Resin was flushed with distilled water and vacuum aspirated, and residues were extracted by sequential elution with 100 ml each of methanol and methylene chloride (8–12 ml/min). Both eluates were reduced to 2 ml, first by rotary evaporation and then with nitrogen gas, combined, and quantified by LSC to determine ^{14}C recovery from the system.

Changes in endogenous phosphate metabolites. Three red abalones (averaging 3 years, 89 g, and 9 cm) were exposed separately to 1.2 mg/l of PCP for 5 hr in flowing seawater at ^{14}C within the NMR magnet. The PCP stock solution was prepared in a 6-l fluoropolymer bag at 8 mg/l, and the final 1.2 mg/l solution for exposure was obtained by combining flows from the bag (6 ml/min) and the carboy (34 ml/min) to produce a total flow of 40 ml/min. A capillary tube filled with a solution of methylene diphosphonate (MDP, the external standard, pH 9.5) was mounted on the surface of the probe opposite the organism, and parallel controls (without PCP) were used to check for any biochemical effects from the system and to measure longitudinal relaxation times (T_1s).

All *in vivo* ^{31}P NMR spectra were acquired by using a copper foil, 3.5 cm in diameter, two-turn spiral surface probe on a General Electric (Fremont, California) CSI-2T spectrometer equipped with a 2-T 200-mm clear-bore horizontal magnet system. For optimum performance with highly conductive seawater systems, the coil portion of the probe was computer-drafted and photoetched (Fan and Higashi, 1989), and the tuning circuitry was balance-matched (Murphy-Boesch and Koretsky, 1983); a typical pseudo-90° pulse for abalone foot with this probe was 30–35 μsec. For time-course studies, one-pulse experiments were carried out with the following spectral conditions: a pulse width of 20–25 μsec, a spectral width of ±1500 Hz, a pulse delay of l sec, and 1000 sample points. Each file was an average of 512 transients, which corresponded to 9.63 min of acquisition time, and relative quantification was obtained by normalizing the various peak intensities to that of the external standard (MDP), which remained constant. Intracellular pH (pH_i) was calculated from the difference in chemical shift between the P_i and PA resonances and a standard calibration curve. For T_1 measurements, a modified inversion-recovery pulse sequence (composite 220°–τ–90°–acquire) was used with similar spectral conditions as the one-pulse experiment except for a 5-sec pulse delay. A total of nine different delay (τ) values were chosen, and for each τ, 256 transients were averaged. The T_1 for each resolved NMR peak was obtained from an exponential curve fitting of the peak intensity vs. τ plot.

Changes in Other Endogenous Metabolites. To determine the kinetics of other important intermediary metabolites, we exposed 15 abalones (averaging 18 months in age, 5 g, and 2.5 cm shell length) to 1.2 mg/l of PCP for 5 hr, and clean water thereafter, at 14°C in an 8-l static glass tank (the solution was renewed hourly); three others in a second tank containing seawater only served as controls. After 0, 2.5, 5, 8, and 21 hr, three abalones were removed, and their foot muscles were dissected, freeze-clamped with liquid nitrogen, and weighed. Dissections were completed in less than 1 min to reduce tumover of important compounds. Perchloric acid (PCA) extractions were

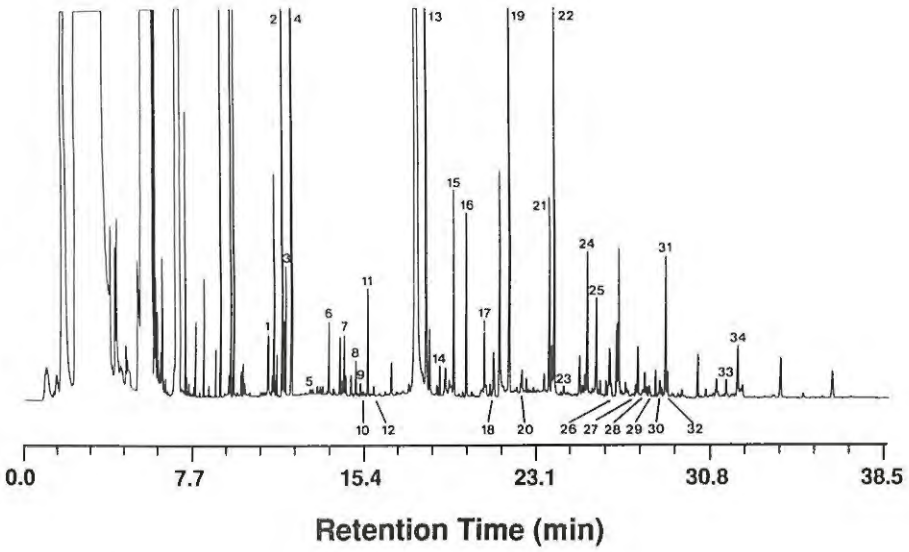

Figure 2. Gas chromatogram (1 cm/min, 65% reduction of original) of an abalone foot muscle extract (conditions are presented in the text): (1) lactate, (2) pyruvate, (3) Ala, (4) Gly, (5) α-aminobutyric acid, (6) Val, (7) Leu, (8) Ile, (9) succinate, (10) γ-aminobutyric acid (GABA), (11) Pro, (12) fumarate, (13) glyceraldehyde 3-phosphate, (14) Met, (15) Ser, (16) Thr, (17) Phe, (18) malate, (19) Asp, (20) hydroxyproline, (21) Glu, (22) ornithine, (23) β-hydroxybutyric acid, (24) Lys, (25) Gln, (26) Arg, (27) glycerol 3-phosphate (a), (28) His, (29) glycerol 3-phosphate (b), (30) citrate, (31) Tyr, (32) Trp (a), (33) ascorbate, (34) Trp (b).

performed by using a procedure modified from that of Gutmann and Wahlefeld (1974). Foot muscle was ground to a fine powder in liquid nitrogen, extracted twice with 5% $HClO_4$ at a ratio of 1:2 (w/v), and centrifuged for 25 min at 27,000 g in a Sorvall refrigerated centrifuge (Dupont Instruments, Newtown, Connecticut). The combined supernatant fluids were titrated to neutrality with KOH and centrifuged at 27,000 g for 25 min. Aliquots were distributed to separate vials, lyophilized, and stored desiccated at –20°C until use.

Analysis was performed by using a method modified from that previously described (Fan et al., 1986). The lyophilized extracts were sonicated for 2 hr, then incubated in pyridine: *N*-methyl-*N*(*t*-butyl-dimethylsilyl)trifluoroacetamide (MTBSTFA, 1:1) overnight at room temperature to yield silylated derivatives. Simultaneous analyses for amino acids, organic acids, and phosphagens (more than 40 components) were performed on each sample by injection of the silylated PCA extract on a Varian (Palo Alto, California) Model 3300 gas chromatograph equipped with a splitless injector, an open-tubular column of DB-1 film 0.4 µm thick (0.18 mm inner diameter x 0.4 mm outer diameter x 40 m long; J&W Scientific, Rancho Cordova, California), and flame ionization detection (GC-FID; Figure 2). Zone temperatures were injector, 260°C; column, held at 60°C for 1.5 min after injection, ramped up to 150°C at 20°C/min, then to 300°C at 6°C/min, where it was held for 5 min; and detector, 320°C. A splitless injector purge of 100 ml/min occurred at 1.0 min after injection, and the hydrogen carrier gas velocity was 60 cm/sec. Identities were confirmed by gas chromatography-mass spectrometry (GC-MS) of authentic standards with a Hewlett-Packard (Palo Alto, California) Model 5890 gas chromatograph, with the same column and conditions, interfaced to a VG Trio 2 mass spectrometer (Manchester, UK).

Project Results

Exposure system. Although a variety of flow-through systems for use in toxicological studies have been reported (Garnas and Crosby, 1979; Tjeerdema and Crosby, 1987), NMR spectroscopy presents unique requirements not addressed by previous designs. The system must circulate large volumes of temperature-controlled, aerated water to an organism within an NMR magnet while closely regulating exposure to the toxicant. Thus, the chamber and perfusion lines must be made of nonmagnetic, nonconductive, chemically inert materials and be visually clear to help in placement of the probe. Also, the system must be of low intrinsic dead volume, as infusion lines must be long to keep the pumps away from the magnet, be rigorously leakproof to avoid damage to the probe, allow electrical grounding and shielding, and present a minimal distance between the organism and surface probe.

Our exposure system met all of these criteria; abalones can be maintained in this system without observable metabolic change for more than 22 hr. Also, use of a flexible fluoropolymer bag for containment allows animals of various sizes and shapes to be used while maintaining minimal dead volume; it has also accommodated marine bivalves without modification (unpublished results).

Toxicity Rangefinding. In the broad-range test, abalones exposed to PCP at ≥2 mg/l died during the 6-hr exposure period, and those exposed to PCP at 1 mg/l died during the recovery period, indicating a 6-hr LC_{50} of 0.5–2 mg/l. In the narrow-range test, all individuals exposed to PCP at ≤0.8 mg/l showed no toxic effects after 6 hr, and all those exposed to PCP at ≥1.6 mg/l appeared dead. However, three abalones exposed to PCP at 1.6 mg/l later recovered, indicating a 6-hr LC_{50} and NOEL of approximately 1.6 and 0.8 mg/l, respectively.

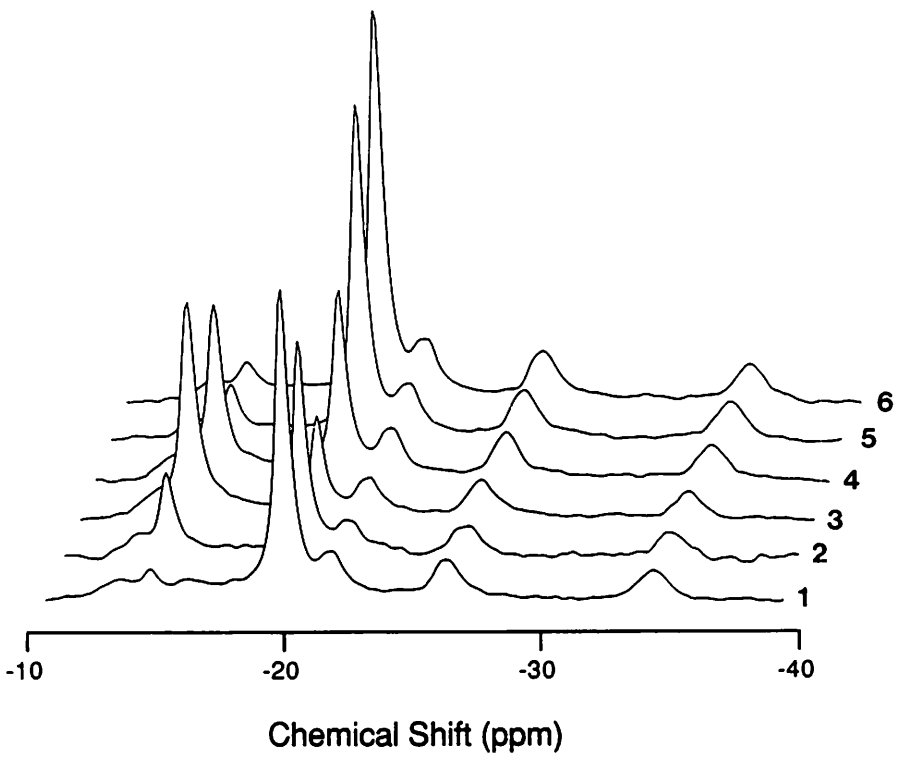

Figure 3. Representative ^{31}P NMR spectra of abalone foot muscle showing a full exposure-recovery time course: (1) preexposure, (3) after 5-hr of exposure to 1.2 mg/l of PCP, and (6) after 13-hr of recovery. A, inorganic phosphate; B, phosphoarginine; C, γ-phosphate of ATP with overlapping of the β-phosphate of ADP; D, α-phosphate of ATP with overlapping of the α-phosphate of ADP and the phosphate of NAD; and E, β-phosphate of ATP.

Therefore, to ensure maximal sublethal responses, we chose an exposure of PCP at 1.2 mg/l for 5 hr for both the disposition and *in vivo* NMR experiments.

PCP disposition. Although the system was designed for aquatic *in vivo* NMR spectroscopy, it was also useful for investigating [^{14}C]PCP disposition; total system ^{14}C recovery was 91.4%, indicating insignificant losses from surface adsorption, resin breakthrough, or other causes. Also, no toxic signs were detected during the vehicle control, and no preexisting radioactive residues were found in foot muscle.

The muscle residue level after 5 hr of exposure to PCP was 25.7 ± 5.5 µg/g (in PCP equivalents), and the 5-hr total concentration factor (TCF) was 21.4 ± 4.6; both represent the combination of PCP and any metabolites responsible for the metabolic effects described in the following sections. The TCF is similar to a bioconcentration factor (Neely et al. 1974; Spacie and Hamelink, 1982), except that it includes both the parent substrate and metabolites and, because of the short time of exposure, does not assume steady-state conditions (Tjeerdema and Crosby, 1987, 1988). Also, it allows comparison with published data obtained by using exposure to different concentrations of PCP. After 13-hr of depuration, the residue level of PCP in the muscle decreased to 5.7 ± 2.7 µg/g, indicating an elimination of 77.8%. Whether PCP was depurated from the body or translocated to other organs, the decreased level coincided temporally with recovery of both endogenous metabolite levels and pH_i, as described in the following sections.

Changes in endogenous phosphate metabolites. Representative ^{31}P NMR spectra for abalone foot muscle during an exposure-recovery sequence are shown in Figure 3. Spectral assignments were guided by previously published spectra for abalone (Burt et al., 1976; Higashi et al., 1988), the general database of muscle constituents observable *in vivo* by ^{31}P NMR (Gadian, 1982), and spectra from standards of P_i, ATP, ADP, PC, PA, glucose 6-phosphate, $MgCl_2$, and NaCl (all at physiological pH; data not shown). Thus, the nucleoside phosphate (NP) peaks labeled α, γ, and β were attributed to αATP + αADP + the phosphate moiety of NAD, γATP + βADP, and βATP, respectively. In addition, close similarity between the *in vivo* and PCA-extract spectra indicated that the *in vivo* spectra represent foot muscle only, with only negligible interference from surrounding tissues (Higashi et al., 1988). The measured T_1 values for PA (3.23 sec), P_i (4.83 sec), and the α- (1.12 sec), β- (1.04 sec), and γ- (1.55 sec) NPs indicated partial saturation of resonances under the conditions used, but this did not affect relative quantification.

Figures 4 through 6 show the detailed changes of constituents over time in foot muscle *in vivo*. Although all animals responded similarly during both exposure to PCP and recovery, data from each abalone (A through C) are presented individually to highlight differences. The ability to reveal subtle variations by normalization to individual response is one of the main advantages of the *in vivo* NMR approach.

The most obvious changes involved the concentrations (in brackets) of PA and P_i (Figure 4). Changes in foot muscle [PA] and [P_i] appeared complementary through both exposure and recovery. During exposure to PCP, while [PA] initially increased 15–20%, and then declined to 60–80% of preexposure levels, [P_i] simultaneously decreased 30-40% and then increased to 300–800% of preexposure levels. The [PA] decline ceased within the first 2 hr of recovery, then returned toward preexposure levels; however, in abalones B and C, preexposure levels were still not attained after 13 hr of recovery (Figures 4B and 4C). The increase in [P_i] also ceased during the first 2 hr of recovery; after 2 hr, it returned to preexposure levels in all three abalones.

Figure 5 presents comparisons of the time courses of [PA] and pH_i. Foot muscle pH_i was 7.3–7.4 before exposure; a slight increase to between pH 7.4 and 7.5 occurred during the first 2 to 4 hr of exposure to PCP. However, in the later exposure and early recovery phases, it became more acidic, to between 7.0 and 7.2. During recovery, pH_i eventually returned to almost the preexposure value in abalone A; in abalones B and C, in which [PA] had not completely recovered after 13 hr, it ultimately increased to more than preexposure levels (Figures 5B and 5C).

Figure 6 presents the time courses of the three NP peaks. From peak assignments, the relative NP concentrations of interest were expressed as follows: [ATP] = β, [ADP] = γ – β, and [NAD] = α – γ. Although peak intensities fluctuated, peak ratios remained steady, indicating that any overall trends were due mainly to changes in [ATP]. In all three abalones, [ATP] appeared to decline slightly during the later part of exposure to PCP. During recovery, it increased more than preexposure levels in abalone A (Figure 6A) and decreased to less than the preexposure levels in the other two (Figures 6A and 6B). An exception was the disproportionate transient increase in the γ-NP peak 7 hr into recovery observed in abalone C (Figure 6C), as it coincided with an increase in [PA] (Figure 4C).

Changes in other endogenous metabolites. Although 32 intermediary metabolites were monitored for changes during both exposure to PCP and recovery periods (Figure 2), only those that significantly changed are presented (Figure 7). Although variations among individuals were observed, the average level of the glycolytic intermediate glyceraldehyde-3-phosphate was approximately 2 µmol/g during exposure to PCP, but in recovery, it declined by more than 50% (Figure 7A). The levels of glycerol-3-phosphate increased from 0.04 to more than 0.20 µmol/g during exposure and then returned to almost preexposure level during recovery (Figure 7A). The fermentation end product lactate increased from 0.5 to more than 6.5 µmol/g during exposure and then returned to almost preexposure levels during recovery (Figure 7B).

The average muscle concentrations of the tricarboxylic acid cycle intermediates citrate, succinate, and malate also increased

during exposure to PCP; [citrate] increased from 0.3 to 0.5 µmol/g (and exhibited significant individual variation), [malate] rose from 0.15 to 0.35 µmol/g, and [succinate] increased from 0.05 to 0.95 µmol/g (Figures 7B and 7C). However, during recovery, whereas malate and succinate concentrations returned to almost preexposure levels, citrate declined to less than preexposure levels (0.1 µmol/g).

Levels of alanine and glutamine also changed significantly during exposure to PCP and recovery (Figure 7D). [Ala] increased from 0.5 to more than 3 µmol/g during exposure, then declined to its preexposure level during recovery. [Gln] remained near 0.6 µmol/g during exposure and then declined to

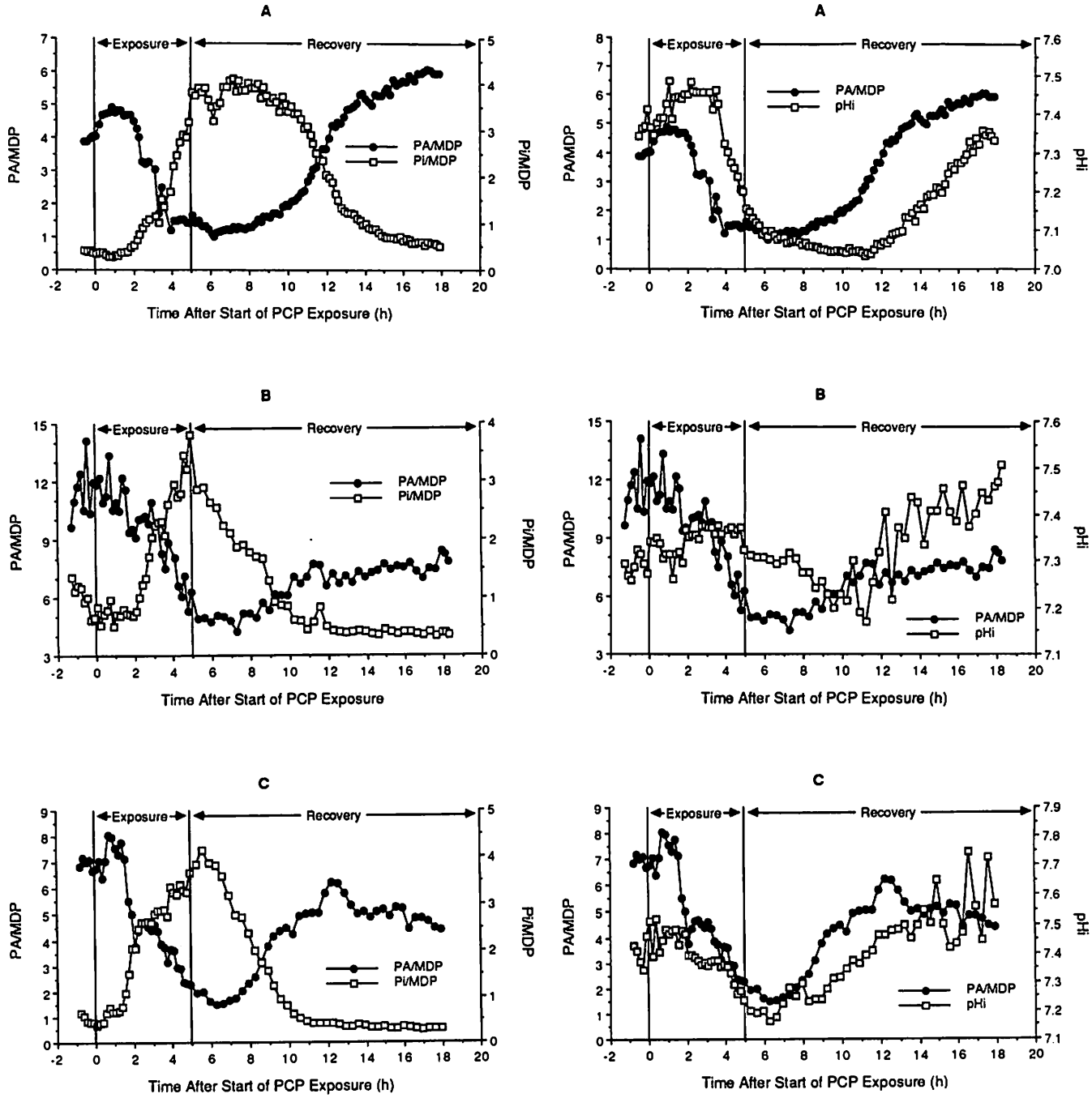

Figure 4. Comparison of the time courses for phosphoarginine (PA) and inorganic phosphate (P_i) in three abalones (A, B, and C) during and after exposure to 1.2 mg/l of PCP. Points indicate changes in peak intensities as related to methylene diphosphonate (MDP), and each point represents 512 spectra averaged over 9.63 min.

Figure 5. Comparison of the time courses for phosphoarginine (PA) and intracellular pH (pH_i) in the three abalones (A, B, and C) during and after exposure to 1.2 mg/l of PCP. Points for PA indicate changes in peak intensities as related to methylene diphosphonate (MDP). Each point (PA and pH_i) represents 512 spectra averaged over 9.63 min.

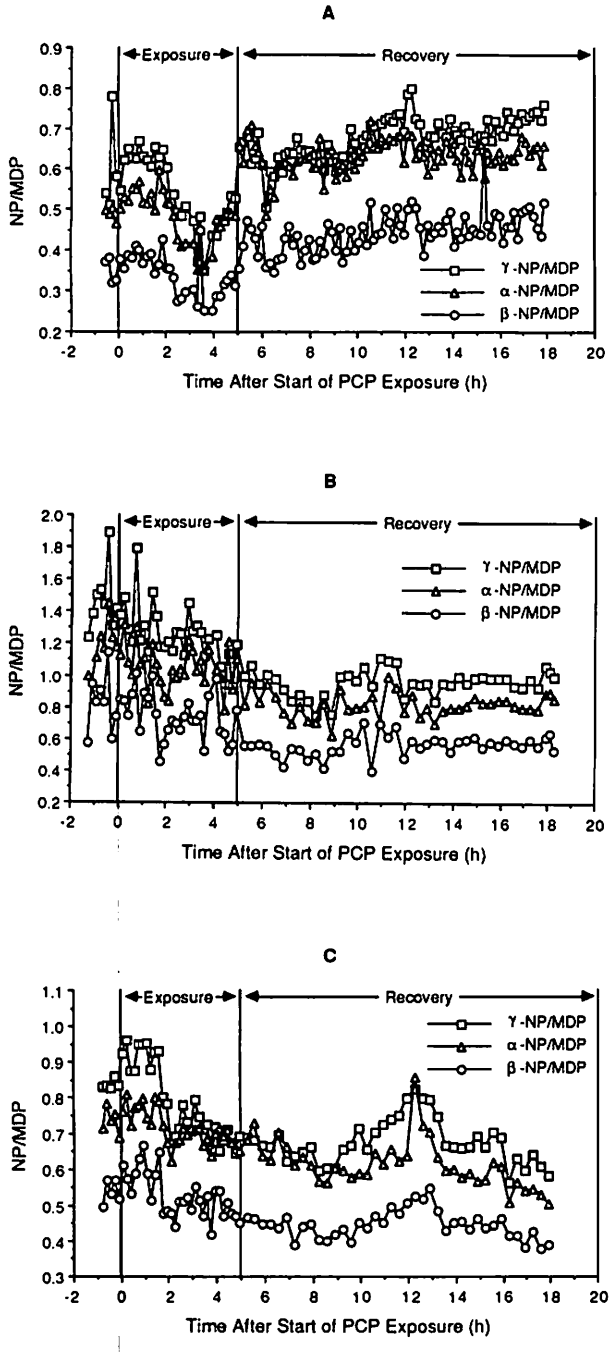

Figure 6. Comparison of the time courses of nucleoside phosphates (NPs) in three abalones (A, B, and C) during and after exposure to 1.2 mg/l of PCP: γ-NP, γ-phosphate of ATP with overlapping of the β-phosphate of ADP; α-NP, α-phosphate of ATP with overlapping of both the α-phosphate of ADP and the phosphate moiety of NAD; and β-NP, β-phosphate of ATP. Points indicate changes in peak intensities as related to methylene diphosphonate (MDP), and each represents 512 spectra averaged over 9.63 min.

less than 0.1 μmol/g during recovery.

Discussion

The toxicity of PCP has been determined for a number of aquatic species. In fish, 96-hr LC_{50}s range from 0.04 mg/l in bluegill (*Lepomis macrochirus*; Johnson and Finley 1978) to 1.7 mg/l in flagfish (*Jordanella floridae*; Fogels and Sprague, 1977); in the rainbow trout (*Oncorhynchus mykiss*, formerly known as *Salmo gairdneri*) alone, it ranges from 0.06 to 0.23 mg/l, depending on conditions (Fogels and Sprague, 1977). For aquatic mollusks, information on toxicity is both less available and just as variable; 96-hr LC_{50}s range from 0.50 mg/l in the whelk (*Thais clavigera*; Nishiuchi, 1977) to 18.0 mg/l in the bay mussel (*Mytilus edulis*; Adema and Vink, 1981). In the giant abalone (*Haliotis gigantea*), PCP toxicity ranges from a 24-hr LC_{50} of 0.85 mg/l to a 96-hr LC_{50} of 0.53 mg/l (Nishiuchi, 1977). The 6-hr LC_{50} for red abalone (1.6 mg/l), though from a limited test, is consistent with those reported for giant abalones.

The 5-hr TCF (21.4) for PCP in red abalone foot muscle was twice that reported (9.9) for the lateral muscle of goldfish (*Carassius auratus*) after 5-hr of exposure to 0.2 mg/l of PCP (Kobayashi and Akitake, 1975). Also, foot muscle was markedly more efficient in clearing PCP residues (78% in 13 hr) than lateral muscle was (30% in 12 hr), possibly because of increased blood flow and/or metabolic conversion of PCP to excretable products. Although biotransformation of PCP by gastropods has not been described, in shortnecked clams (*Tapes philippinarum*), it is detoxified by sulfation (Kobayashi, 1978).

The use of ^{31}P NMR spectroscopy, GC-FID, and GC-MS allowed a number of sublethal metabolic effects from PCP to be observed. NMR spectroscopy reveals only "free" metabolites, so changes in peak intensities could also arise from changes in metabolite mobilization, as with ADP and P_i during ischemia (Gadian, 1982). Also, changes in the chemical shift of P_i, although normally attributed to changes in pH_i, may be affected by other factors,

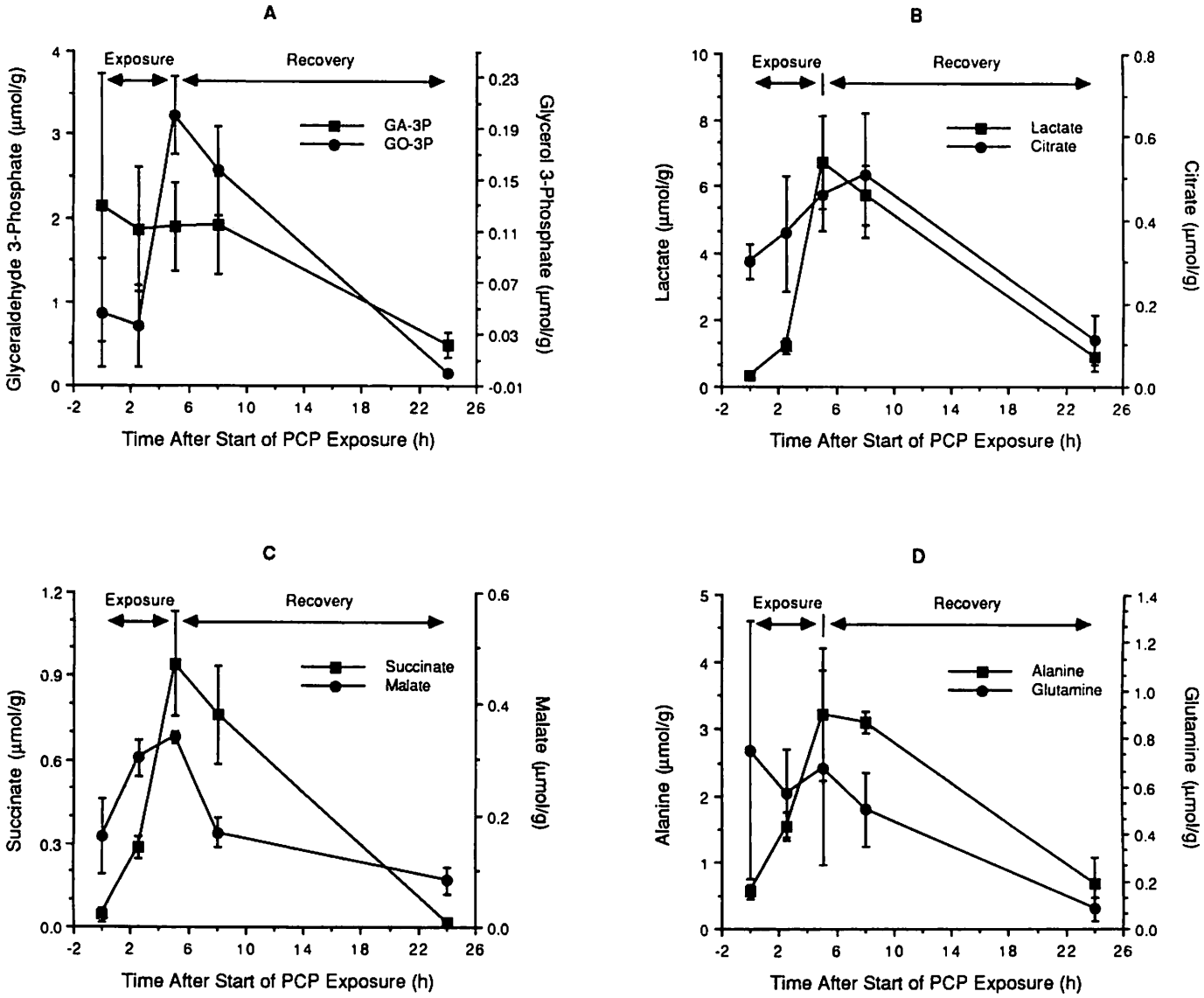

Figure 7. Time courses of other important intermediary metabolites in abalones both during and after exposure to 1.2 mg/l of PCP; bars represent standard deviation (n = 3).

such as the composition of intracellular free ions (Gadian, 1982). However, because of a lack of evidence, we have followed convention in assuming that changes in P_i chemical shift are primarily due to those in pH_i.

Overall, the metabolic effects of PCP coincided with its uptake and depuration. In addition, changes in pH_i and concentrations of PA, ATP, and glycerol 3-phosphate were delayed by about 2 hr, possibly reflecting the time required for PCP uptake and distribution to foot muscle. The inverse changes in [PA] and [P_i] induced by exposure to PCP are consistent with both the blockage of mitochondrial ATP synthesis and ATP replenishment at the expense of PA. Inhibition of mitochondrial ATP synthesis is consistent with accumulation of several TCA cycle intermediates (succinate, malate, and citrate) and glycolytic products (lactate, alanine, and glycerol 3-phosphate). The increase in TCA cycle intermediates can occur by feedback inhibition of the TCA cycle (from the lack of mitochondrial ATP synthesis), whereas accumulation of glycolytic end products can occur as a result of basal ATP production by glycolysis. Also, changes in pH_i, although reflecting changes in the energy metabolism of phosphates, cannot be used alone for metabolic interpretation. Whereas both glycolysis and ATP utilization result in acidification, conversion of PA to maintain ATP levels results in the opposite (Zubay, 1988). Therefore, effects must first be characterized by changes in phosphate metabolites; concurrent changes in pH_i reflect both these and other effects.

The responses were similar to those shown previously by NMR spectroscopy in excised tissues and intact organisms under environmental or functional hypoxia.

In the muscles of cockles (*Tapes watlingi*; Barrow et al., 1980), bay (*Mytilus edulis*) and ribbed (*Geukensia demissa*) mussels (Ellington, 1983), barnacles (*Balanus nubilis*; Dubyak and Scarpa, 1983), and crayfish (*Orconectes virilis*; Butler et al., 1985) exposed to hypoxic conditions, [PA], [P_i], and pH_i showed qualitatively similar responses. In lateral muscles of intact carp (*Cyprinus carpio*) and goldfish (*C. auratus*), similar changes occurred, except that PC replaced PA as the phosphagen (van den Thillart, et al., 1989). The muscles of prawns (*Palaemon elegans*; Thebault et al., 1987) and shrimp (*Crangon crangon*; Kamp and Juretschke, 1987) responded to electrical stimulation with a rapid decrease in [PA], increases in [P_i] and sugar phosphates, and a decline in pH_i; [ATP] significantly changed in only carp and goldfish. Under hypoxia, both accumulation of anaerobic fermentation products and acidification of pH_i are thought to result from continued operation of glycolysis in the absence of mitochondrial oxidative phosphorylation, and phosphagen hydrolysis serves to maintain [ATP] at near-normal levels (Shoubridge and Radda, 1984; Zubay, 1988). However, a somewhat different response was observed in red and black (*Haliotis cracherodii*) abalones exposed to hypersaline (51 ppt) water. Whereas [PA] declined and [P_i] increased, [ATP] declined by as much as 80%, and pH_i did not change. Responses to hypersalinity may be related to increased demand for osmotic regulation by ATP-dependent mechanisms (Zubay, 1988). Because oxygen is abundant, glycolysis, the TCA cycle, and mitochondrial oxidative phosphorylation are presumably functioning; thus, no significant intracellular acidification occurred.

The response of abalone foot muscle to PCP contains components of both types of hypoxia and osmotic stress. PCP is considered to be an uncoupler of mitochondrial oxidative phosphorylation, and its effects also showed characteristics similar to those of an inhibitor of electron transport. A biocide similar to PCP, 2-hydroxybiphenyl, inhibits transport near coenzyme Q (ubiquinone) (Oelze and Kamen, 1975; Oelze et al., 1978). In addition, because [ATP] began to decline at roughly the same time as [PA], it may also inhibit arginine kinase and/or stimulate increased ATP utilization. In muscles from cockles (Barrow et al., 1980) and barnacles (Dubyak and Scarpa, 1983) exposed simultaneously to both inhibitors of mitochondrial electron transport and arginine kinase, [ATP] began decreasing before most PA was consumed. However, when barnacle muscles were exposed to an electron transport inhibitor (cyanide) only, the phosphagen response was not different from that seen with hypoxia alone (pH_i was not reported; Dubyak and Scarpa, 1983). Inhibition of arginine kinase may account for the only partial recovery of [PA] during the recovery period.

This report describes toxicological onset and recovery processes in a live, intact aquatic animal as measured primarily by *in vivo* NMR spectroscopy and by using a newly developed exposure system. The first two stated project goals have been successfully met; the third is currently under investigation, as we felt measurement of both PCP disposition in foot muscle and the changes induced in other intermediary metabolites was needed more urgently to delineate further the effects observed in meeting the second objective. *In vivo* NMR spectroscopy is a major new approach for the investigation of sublethal toxic effects in aquatic organisms. Biochemical processes now can be measured in live, intact organisms as the processes occur, allowing repetitive multicompound analyses in a single, live organism. Additionally, all responses are effectively normalized to an individual for greater interpretability of interactive effects, and to circumvent large individual variations.

In vivo NMR spectroscopy also presents a more sensitive alternative to many sublethal measurements currently used in aquatic toxicology, and it can be used to measure key parameters that cannot be measured with conventional techniques, such as pH_i. As was evident in this investigation, abalones were seriously affected during sublethal exposure to PCP and not fully recovered 13 hr later, although visually they appeared to be unstressed. The combination of live, intact organisms; appropriate exposure chambers; and *in vivo* NMR spectroscopy can help in determining molecular mechanisms of toxicity under a variety of environmental conditions. We plan to assess (1) the usefulness of *in vivo* NMR with other marine invertebrates, including bivalve mollusks, and (2) its ability to measure the interactive effects of marine pollutants and natural stress factors, including changing water temperatures and hypoxia from emergence.

Cooperating Organizations

California Department of Fish and Game
University of California, Davis, NMR Facility and Department of Environmental Toxicology

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Slumping and Sediment Liquefaction at the Head of Monterey Canyon

San Jose State University
Moss Landing Marine Laboratories
R/NP-1-19B
Project Initiated: January 1, 1990
Project Completed: December 31, 1990

Michael T. Ledbetter

The Loma Prieta earthquake caused severe shaking of the beach, estuarine, and fluvial sediments under the "spit" at Moss Landing. The resulting liquefaction of those sediments led to the destruction of the buildings of Moss Landing Marine Laboratories (Greene et al., 1991) and other structures (Tuttle et al., 1990). The objective of this study was to extend the results of a preliminary study (Greene et al., 1990) of the offshore evidence for earthquake damage in the coastal zone and at the head of the Monterey Canyon (Figure 1). Sidescan sonographs of the nearshore region and the head of Monterey canyon revealed features indicative of sediment movement both from liquefaction of buried sediments and slumping of canyon walls during the quake.

Nearshore Liquefaction

Sediment in the nearshore region experienced the same violent shaking as sediment onshore and apparently liquefied as well. Sonographs of the seafloor revealed small features on the scale of sand boils that were observed onshore (Figure 2). Repeated surveys of the area showed that these features disappeared within two months of the earthquake as a result of normal wave action. The ephemeral nature of these features and their size and location indicate that buried sediment was liquified and brought to the surface. A much larger feature was found in the same area and is clearly an artifact of widescale liquefaction of nearshore sediments. A deformation front 200–400 m long (Figure 2) was identified in water 9–11 m deep in Area 1A (see Figure 1). Large (tens of meters) sediment lobes appear to emanate from the deformation front (Figure 2). When the bathymetric findings are plotted, it is apparent that the deformation front represents the location of a shallow, buried, horizontal sand layer that liquefied during the quake and sent lobes of fluidized sediment into the nearshore zone. The most prominent of these zones corresponds in depth to an onshore sand layer that has been identified as the liquefaction horizon that was responsible for destruction

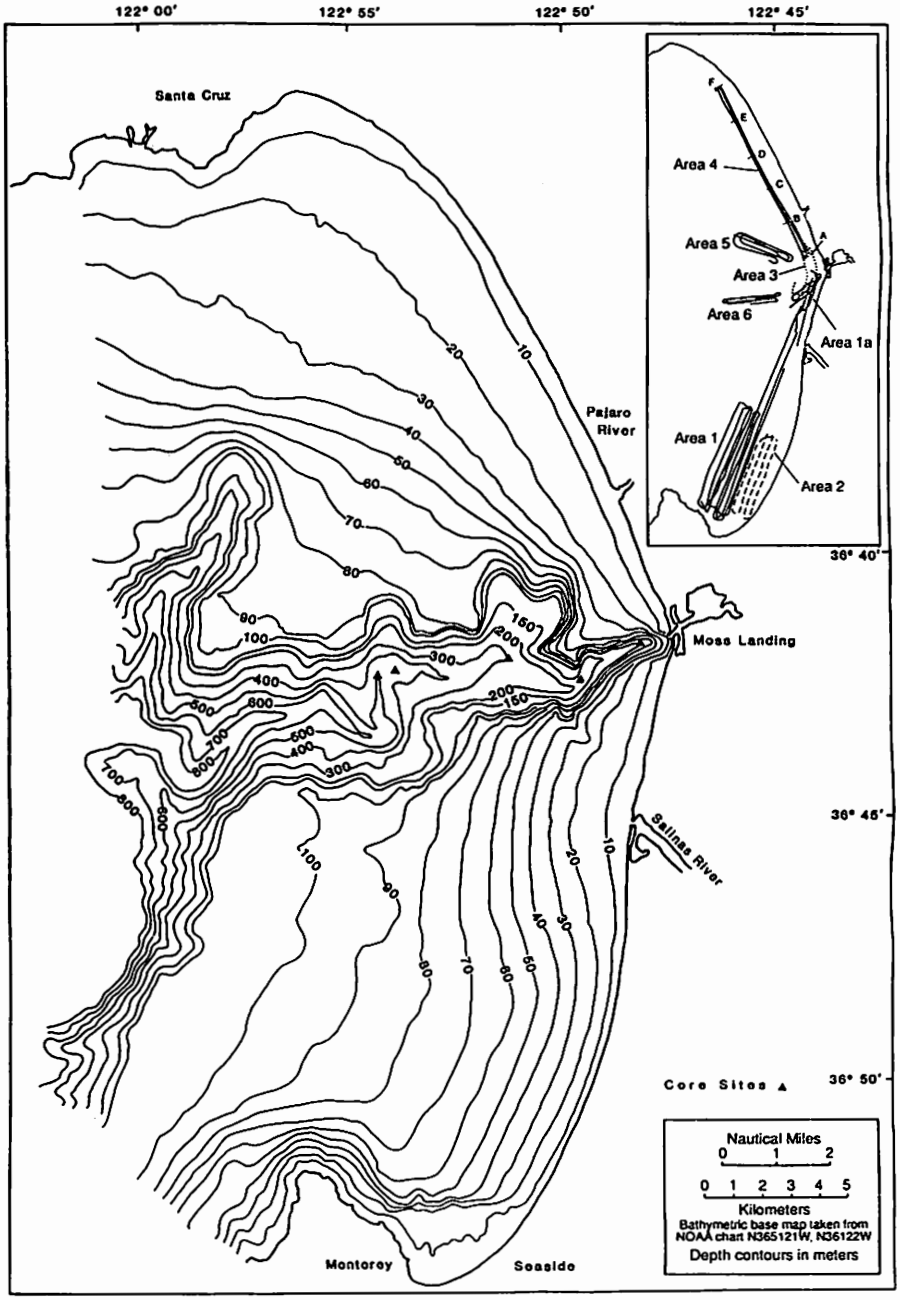

Figure 1. Bathymetric map of Monterey Bay; region studied includes areas 1a and 3 shown on inset.

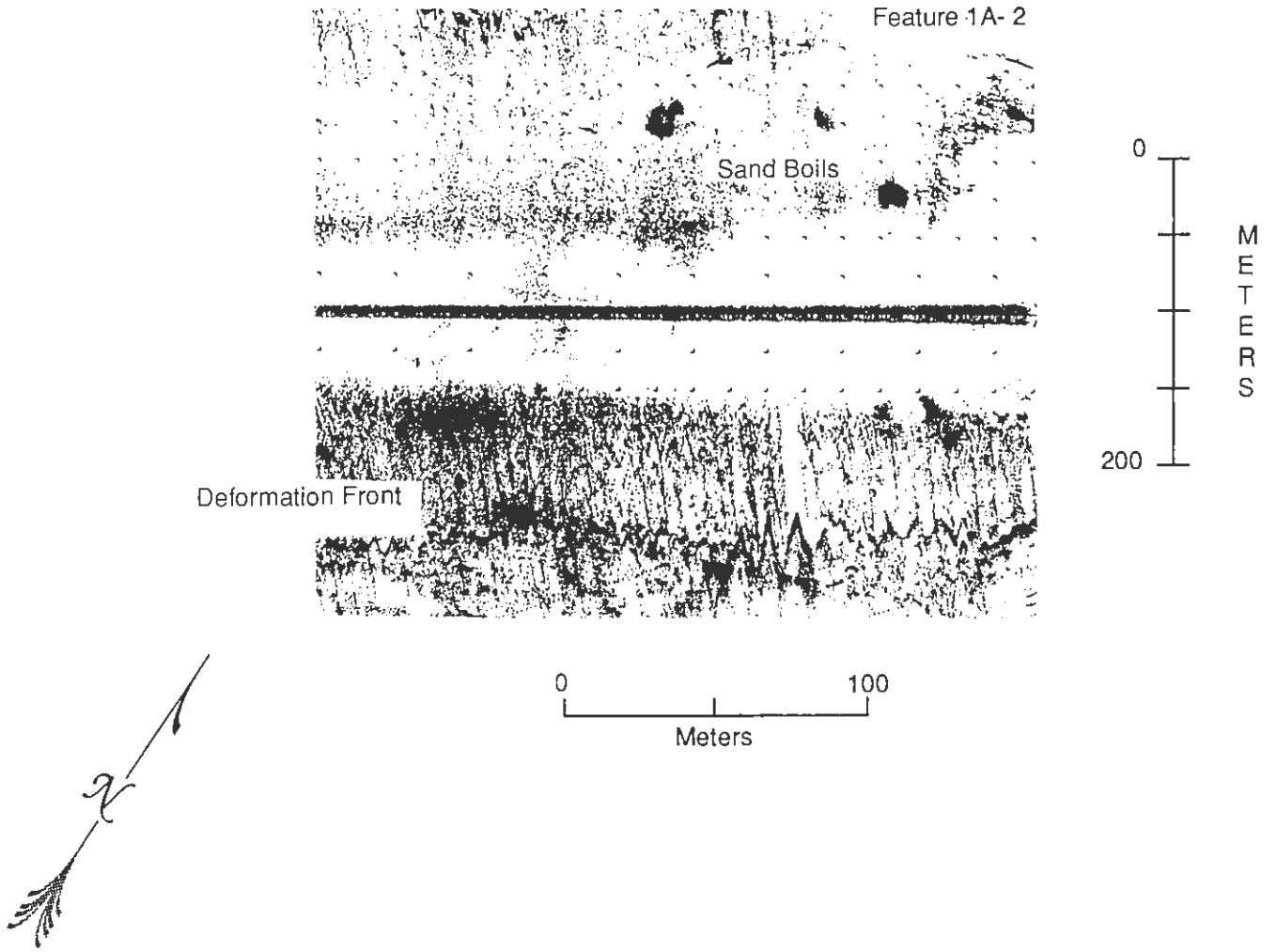

Figure 2. Example of sidescan sonar data. The nearly east-west deformation front represents a zone of liquefied sediment that flowed onto the seafloor. The water depth of the front and associated sediment lobes is 10–12 m and corresponds to the depth of the onshore liquefaction layer.

of the Moss Landing Marine Laboratories buildings (Woodward-Clyde Consultants, 1990). Therefore, the liquefaction horizon flowed horizontally toward the free face represented by the beach face and resulted in an offshore flow of shallow, buried sediment into the nearshore.

Canyon Slumping

Sidescan sonographs, visual inspection via a remotely operated vehicle and diver observations, and a bathymetric survey all show widescale slumping of sediment from the south wall of Monterey Canyon (Greene et al., 1991). Scarps on the wall of the canyon and displaced boulders and slump blocks all indicate that the unstable sediment on the walls at the head of the canyon was shaken loose by the quake. The largest scarp observed was approximately 7 m high at the upper edge of the canyon, and the slumped material was seen on the lower flanks of the canyon. The fresh scarp of exposed sediment was the site of increased benthic faunal activity, and a dense population of fish appeared to be feeding on the newly exposed organic matter at the edge of the canyon (Schwing et al., 1990).

A bathymetric survey of the upper canyon was run along track lines originally surveyed in 1983. All lines showed a canyon axis that is a few meters more shallow than it was on previous surveys; one line showed a slump block 25–30 m thick in the axis near the canyon head at a depth of 175 m (Figure 3). These slump blocks have persisted in the canyon axis for 15 months after the earthquake and may not be flushed down the canyon until normal winter storm conditions return to the northern California coast. In the meantime, some of the slump blocks have revealed large fish populations that may provide a source for a continuous stock of some heavily fished species in the bay (M. Yoklavich, personal communication).

Discovery of a Buried Canyon Head

In the initial survey done 10 days after the earthquake, a shallow seismic reflection survey showed a buried canyon beneath the seafloor south of the present canyon (Figure 4). Initially, it was suspected that the canyon fill might contain porous sediments that were subject to liquefaction. If so, the location of the buried canyon might delineate the

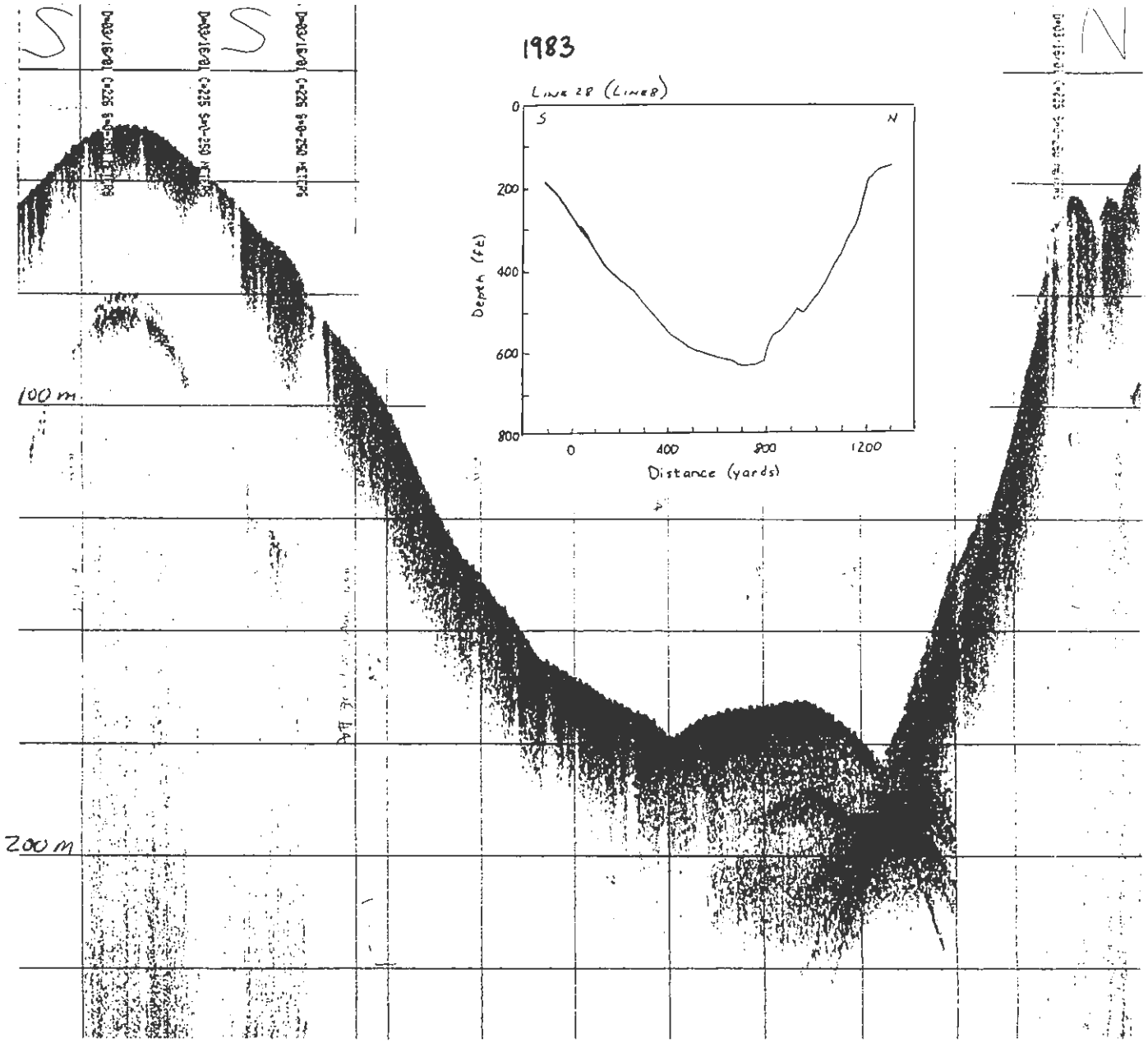

Figure 3. Bathymetric profile of the Monterey Canyon axis near the head of the canyon shows a mound of sediment filling the axis that did not exist on an earlier survey (see inset). The mound in the axis is slump material that slid into the canyon from the upper walls where fresh slump scarps were observed with a remotely operated vehicle.

area of most intense damage and affect reconstruction plans for damaged structures on the coast. Therefore, a seismic survey of the region was conducted in order to trace the buried canyon toward shore.

The surveys showed that the buried canyon is actually a bifurcated canyon much like the present Monterey Canyon head. The nearshore location of the canyon is south of the damaged marine laboratories buildings and north of the present Salinas River. Onshore the canyon is 20–40 m deep and is likely too deep for the canyon fill material to be a factor in liquefaction damage from an earthquake.

Study Ramifications

Clearly, for any structures in the nearshore region, the type of buried sediment on which foundation are erected must be considered. In the case of Moss Landing Marine Laboratories, a slab and pier type of foundation was inadequate to mitigate the spreading of the site due to liquefaction during the Loma Prieta earthquake. (In the 1906 quake, all buildings adjacent to the beach in Moss Landing were destroyed [Lawson, 1908].) The Monterey Bay Aquarium Research Institute marine operations building in Moss Landing was unaffected by the liquefaction that occurred both north and south of the building. That building was reportedly constructed on denser soils (Woodward-Clyde Consultants, 1990) than exist at the marine

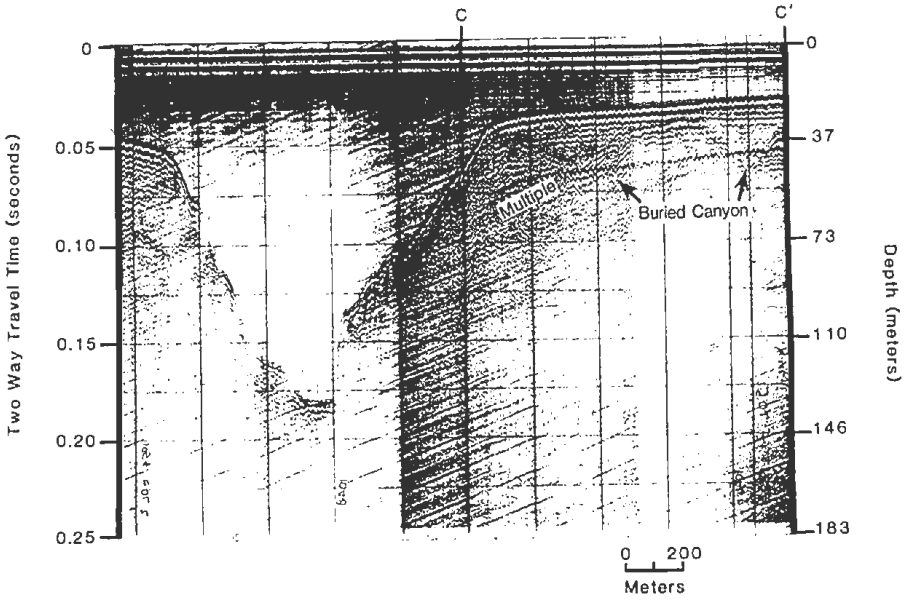

Figure 4. A seismic profile of the southern flank of the Monterey Canyon shows a bifurcated buried canyon that can be traced onshore to a point south of the former location of Moss Landing Marine Laboratories.

laboratory site, and the pier was erected on pilings driven into the harbor. Therefore, a combination of building design and soil conditions may have averted damage at that site. In the future, however, the potential role of liquefaction of loosely consolidated nearshore soils must be taken into account if earthquake hazards are to be mitigated in coastal regions.

The widespread extent of liquefaction and slumping offshore at Moss Landing and adjacent areas also resulted in damage to structures. The outfall of the National Refractories plant at Moss Landing and the sewage outfall for the town of Marina were both severed because of the earthquake. The relative role of slumping and liquefaction is not clearly known in the case of those damaged structures, but, clearly, more thought needs to go into constructing offshore structures now that the widespread nature of offshore liquefaction can be demonstrated.

Cooperating Organizations

Monterey Bay Aquarium Research Institute, Pacific Grove
National Science Foundation
Naval Postgraduate School, Geodesy Department, Monterey, California
U.S. Geological Survey, Pacific Marine Geology Branch, Menlo Park

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University of California, Los Angeles Marine Science Center

University of California, Los Angeles
R/NP-1-19E
Project Initiated: May 1, 1990
Project Completed: September 30, 1991

William M. Hamner

Introduction

Research or instruction in marine sciences traditionally has not been considered as a major focus of activity at UC Los Angeles (UCLA). Nonetheless, over the years, interest in the marine environment has quietly grown to the point that some 25 principal investigators at UCLA are now engaged in research that is partly or entirely marine oriented. Further, more than 50 courses are now offered each year on this campus on aspects of marine science, taught independently by five departments. In recognition of the true strengths in both teaching and research in the marine sciences at UCLA, the campus administration authorized in June 1989 the formation of the UCLA Marine Science Center. Dr. William Hamner, Department of Biology, is director of the center and Drs. Chapman (Biology), Orme (Geography), and Turco (Atmospheric Sciences) serve on the executive committee. The project has allowed the center to hire a half-time administrative assistant, level III, to work with the Director at the center.

Project Objectives

The objectives of the proposal have been to increase the administrative effectiveness of the new UCLA Marine Science Center. The timetable was March 1, 1990, to June 30, 1991. The center has initiated and is continuing to develop a privately funded support group of UCLA alumni and friends of the university, which hereafter will provide additional resources for research and education in the marine sciences at UCLA, as well as for administrative assistance.

Work to Date

The new Marine Science Center has already established an interdepartmental association of senior and junior faculty members, research associates, and graduate students interested in the marine environment. In the 1990 winter quarter, the association met regularly for a weekly seminar series on aspects of marine science, with the senior faculty giving the first 15 seminars. The series was well attended and was interdisciplinary in its approach. A similar series was offered during the winter quarter 1990-1991.

The Center sponsored a presentation on May 6, 1990, at the Balboa Bay Yacht Club in Newport Beach, California, which was hosted by an interested private citizen who has also made a donation to the center. The event was instrumental in initiating the privately funded support group for the Center. Follow-up with the contacts made through alumni and the lay public who attended this function continues, and plans for a second such event are in development.

In addition, the Center submitted to the Academic Senate of the UCLA campus a proposal for an interdisciplinary Ph.D. program on global geo-biosphere dynamics, established a new undergraduate major with a marine biology concentration, and completed its first edition of a biannual newsletter. Finally, the Center hired an administrative assistant on a half-time basis. This person is handling and processing the paperwork, filing, and correspondence and doing other routine administrative tasks, thus relieving the Director of these duties. Additionally, she has been assisting in the coordination of the activities of the Center with the UCLA National Center for Intermedia Transport Research and has assisted the Director in coordinating meetings with the Los Angeles Unified School District Marine Science Consortium.

Summary

The initial enthusiasm for the Marine Science Center has created an organization that appears to be growing far more rapidly than was initially anticipated.

The Marine Science Seminar Series for the winter quarter 1990–1991 began January 1991. The first phase of the curriculum revision project has been completed with the approval of the undergraduate major with a marine biology concentration. The second phase, approval of the proposed Ph.D. program in global geo-biosphere dynamics, is pending response from the Academic Senate.

Maintenance of Water Balance in Seawater-Adapted Coho Salmon

Humboldt State University
R/NP-1-19G
Project Initiated: October 1, 1989
Project Completed: September 30, 1990

Theodore H. Kerstetter

Background

Marine teleost fish live in an environment that, because of its high salinity, continuously draws water osmotically from the fish. Water loss occurs chiefly from the gill, and to replace the loss, a rate of water absorption equal to the loss occurs via the digestive system. Absorption of seawater from the gut into the blood is linked to active (energy requiring) absorption of ions, mainly sodium, potassium, and chloride, and to alkalinization of the intestinal fluid by the addition of bicarbonate ions (Skadhauge, 1974; Ando et al. 1975; Ando, 1980; Ando and Subramanyam, 1990).

Absorption of ions and water must function efficiently to maintain body-fluid homeostasis. If absorption is impaired, or if the mechanisms are not fully functional, dehydration and/or salt loading will result. In theory, two separate dysfunctions are possible: (1) suboptimal volume absorption and (2) an extremely hypersaline absorbate. The second condition would affect the liver in particular, because intestinal absorbate is carried directly to that organ by the hepatic portal system; consequently, the osmotic concentration of the blood perfusing that organ would be abnormally high. (Blood is "desalted" in its passage through the gill, so other internal organs do not face the same osmotic challenge.)

That a hypersaline absorbate can and does occur is indicated by increases up to 20% in the potassium content of liver cells when juvenile salmonids are first transferred to seawater (Kerstetter and Krueger, 1989). Increasing their potassium concentration is one method by which cells maintain volume in the face of extracellular increases in osmolarity (reviewed by Hoffman and Simonsen, 1989). Although no direct evidence indicates that this is harmful, it is commonly accepted in the aquaculture industry that best growth and lowest mortality of juvenile salmon occur when they are transferred to seawater in the "smolting window," the period when they are physiologically preadapted to enter saltwater. Not coincidentally, smolts have higher rates of intestinal water absorption than presmolts (Collie and Bern, 1982) and less extreme rises in liver potassium, when entering seawater (Kerstetter and Krueger, 1989).

The general objective of this project was to examine indirectly some of the changes that occur in intestinal water-absorption mechanisms when juvenile coho salmon go into saltwater. The specific objectives were (1) to characterize changes in the ion composition and pH of intestinal fluid during the first 7 days of seawater (SW) residence and (2) to determine changes in the activity of a key intestinal enzyme implicated in water absorption, alkaline phosphatase (Oide, 1974; Gasser and Kirschner, 1987), also during the first 7 days in seawater. During the period of this research, presmolts were not available, so the measurements were made on smolts, postsmolts, and long-term (>60 days) SW residents (as a point of comparison). For the last two categories, analysis of gut fluid was done for two or more segments. Fish used in the study were aged 15 to 17 months, hereafter referred to as 1-plus.

Laboratory Analyses

Concentrations of sodium and chloride in gut fluid were determined by standard laboratory methods, flame emission spectrometry for sodium and a Cotlove chloridometer for chloride. Because of the small volumes available for analysis, pH of gut fluid was measured with the pH electrode of a micro blood gas analyser linked to a research-grade pH meter, Orion model 550. Alkaline phosphatase was extracted from intestinal mucosa by scraping the mucosa from the lumen-facing side of the intestine, homogenizing it in 10 volumes of cold 0.30 M sucrose, 1.0% Triton X-100, pH 7.60, and centrifuging the homogenate at 20,000 x g for 20 min. The enzyme was assayed as described by Gasser and Kirschner (1987).

Modifications of Intestinal Fluid

Long-term SW residents and smolts after their third day in seawater had lower concentrations of sodium in their intestinal fluid than postsmolts did after 7 SW days (Figures 1–3). Because abundant evidence points to the linkage of ion and water absorption in the teleost gut, it can be concluded that water absorption in the postsmolt intestine is suboptimal, at least for the first 7 days after SW transfer. Sodium concentrations in the luminal fluid from long-term SW residents (Figure 1) showed a greater than 10-fold decrease from stomach (more than 300 mM) to the mid intestine (less than 25 mM), evidence for rapid absorption of monovalent ions in the

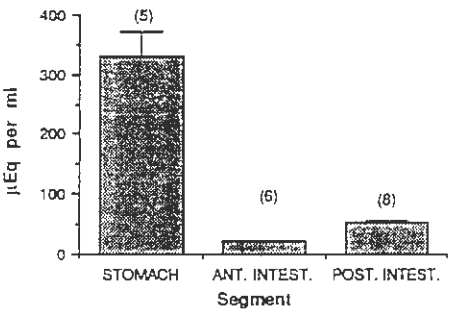

Figure 1. Sodium concentration of luminal fluid taken from three regions of the digestive tract in seawater-adapted, age 1-plus coho salmon. Anterior intestine (ANT. INTEST.) includes that portion from the pyloric valve to the beginning of the large posterior segment. Number of observations is in parentheses above each column. POST. INTEST. = posterior intestine.

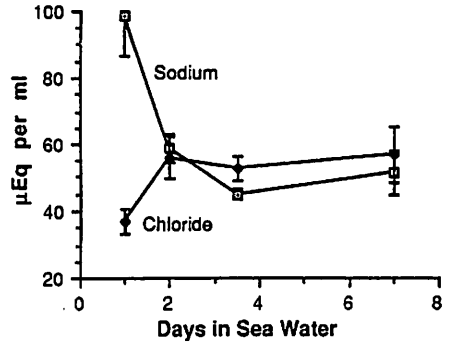

Figure 2. Sodium and chloride concentrations of rectal fluid from age 1-plus coho salmon transferred to seawater in June. For both ions, concentrations on day 1 were significantly different from concentrations on days 2, 3.5, and 7. Each point is the mean of six observations.

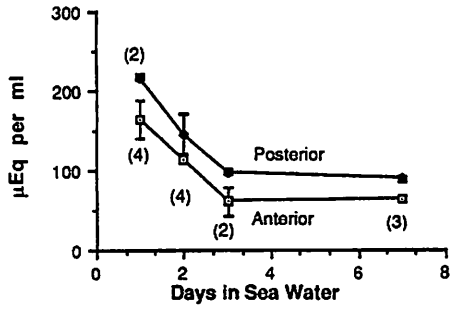

Figure 3. Decrease in the sodium concentration of intestinal fluid of postsmolts transferred to seawater in August. Fluid from the anterior segment was consistently lower in sodium than fluid from the posterior. Compare with Figure 1. In both segments of the intestine, the change was statistically significant by ANOVA. *N* for each pair of samples is in parentheses, except individually for day 1.

anterior regions of the intestine.

The alkalinity of intestinal fluid in marine teleosts has been reported by Shehadeh and Gordon (1969), Dixon and Loretz (1986), and others. The measurement of intestinal fluid pH in this study extends our knowledge in two ways: first, by showing progressive alkalinization of luminal fluid from anterior to posterior, and second, by showing that outside the smolting window (in this case postsmolts), intestinal fluid reaches its high pH (≥8.5) only after several days of SW residence (Figure 4). In contrast, in full smolts, transferred to seawater in June, the pH of intestinal (rectal) fluid was high (8.56 ± 0.04) at the time of the first sample, 24 hours after transfer (Figure 5). The pH of the rectal fluid of nine long-term SW residents was 8.59 ± 0.04.

Intestinal Alkaline Phosphatase

Intestinal alkaline phosphatase activity in postsmolts was low after 24 hours in seawater but increased to values exceeding those of the long-term SW residents by day 7 (Figure 6). Preliminary unpublished work indicates that the activity of the enzyme in smolts 24 hours after SW transfer is at or near levels seen in long-term SW-adapted, age 1-plus salmon. Thus, in coho salmon, increased activity of intestinal alkaline phosphatase appears to accompany adaptation to seawater, or preadaptation in the case of smolts. The particular role of alkaline phosphatase in water absorption is unknown, but when it is inhibited in the intestine of eels, *Anguilla japonica*, and rainbow trout, water absorption is reduced or stopped (Oide, 1973; Gasser and Kirschner, 1987).

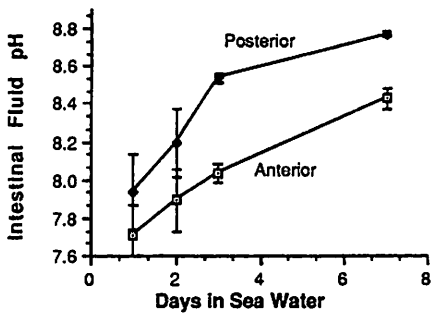

Figure 4. Increases in pH of intestinal fluid of postsmolts during 1 week of adaptation to seawater. Changes over time and by segment are both statistically significant by two-way ANOVA. *N* as in Figure 3.

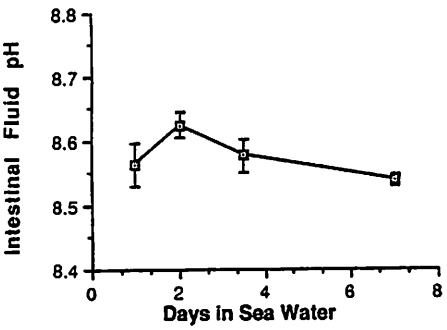

Figure 5. pH of intestinal fluid of fully smolted coho salmon during 1 week of adaptation to seawater. Compare with Figure 4. *N* = 6 for each sample.

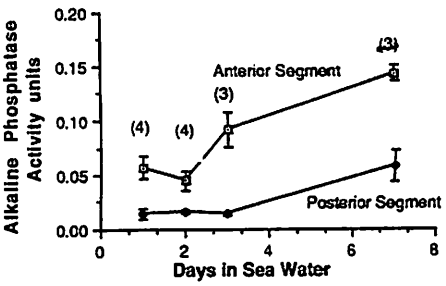

Figure 6. Increase in intestinal alkaline phosphatase activity in postsmolts during 1 week of adaptation to seawater. Units are micromoles of substrate hydrolyzed per minute per milligram of mucosa. Note that the enzyme is three to four times more concentrated in the mucosa of the anterior segment. Number of observations for each sample pair is in parentheses.

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Kerstetter, T. H., and R. J. White. Adaptation of coho salmon to saltwater: A developmental study of intestinal water absorption. To be presented at the 4th International Workshop on Smolt transformation, October 1992.

Accelerating the Development of Ecosystem Functions in Restored and Constructed Wetlands

San Diego State University
R/NP/1/191
Project Initiated: June 1, 1990
Project Completed: September 30, 1990

Joy B. Zedler and René Langis

Introduction

In a review for the Environmental Protection Agency (Kusler and Kentula, 1989), scientists from around the United States concluded that the science of wetland restoration is in its infancy. Three studies of constructed salt marshes—in North Carolina, Texas, and southern California—revealed a common problem, namely low organic content in sediments as compared with sediments in natural reference wetlands (Table 1).

Sediments at the natural Texas sites (Lindau and Hossner, 1981) were low in both organic carbon (<1.3%) and nutrients (total nitrogen, <600 mg/kg), but levels in the constructed marsh were even lower (<0.5% and <100 mg/kg, respectively). In most of the natural marshes studied in North Carolina (Craft et al., 1986, 1988), the concentrations of organic carbon and nutrients were high (organic carbon as high as 8.6% and total nitrogen up to 1680 mg/kg), but the constructed wetlands had lower levels of organic matter and nutrients. The same pattern was shown in southern California (Langis et al., in press); a constructed marsh in San Diego Bay had lower levels of nitrogen and organic carbon than the adjacent natural marsh (see Table 1).

Organic sediments are a basic feature of natural wetlands—they influence nearly every aspect of the functioning of the wetland ecosystem by changing sediment porosity, water-holding capacity, nutrient dynamics, plant growth rates, and foliar nutrient quality and by influencing the species composition and abundance of invertebrates associated with the sediments. The microbes, plants, and animals, in turn, affect the rate of accumulation of organic matter in wetland sediments.

In this project, we tested the hypothesis that low levels of organic matter and associated nitrogen slow the rates of functional development. We sampled the San Diego Bay marsh at age 6 years to determine if conditions had improved through time, and we began a field experiment to test how soil amendments would improve soil and plant development. We predicted that the rate of ecosystem maturation for newly constructed wetlands would be accelerated by augmenting the amount of organic matter and nitrogen in the sediment.

Results and Discussion

Long-term comparisons. Soils and vegetation at the Connector Marsh (CM) and the natural Paradise Creek Marsh (PC) were compared in the summer of 1990, 6 years after CM had been constructed. Although cordgrass biomass was higher this year than in the past, two differences between the constructed and natural marshes persisted: Cordgrass at CM had only 60% of the biomass of cordgrass at PC (Table 2), and few plants at CM were tall (Figure 1).

Within the constructed marsh, one sampling station stands out as functionally impaired. At station NI 4, cordgrass density, biomass, and height were all low in 1990. This was the only site that did not show an increase in biomass between 1989 and 1990. The cause of the poor growth appeared to be an outbreak of scale insects (*Heliaspis spartina*), a native, host-specific herbivore. At the San Diego Bay dredge-spoil island, this same scale insect has nearly eliminated the cordgrass population that was transplanted several years ago. These insect outbreaks appear to occur where native predators are rare (e.g., the omnivorous beetle, *Coleomegilla fuscilabris*; K. Williams, San Diego State University, personal communication). The number of beetles, in turn, appears to be low where cordgrass vegetation is short because these insects are terrestrial arthropods and benefit from high-tide refuges among tall vegetation. Thus, where plants are short, beetles are at a disadvantage, and scale populations can explode, further impairing plant growth and keeping the canopy short. Such a cycle is not easily broken when cordgrass occurs in isolated patches, as in the islands of the Connector Marsh.

Field experimentation. The California Department of Transportation completed excavation of a 17-acre intertidal wetland on February 27, 1990. In early March, just before it was opened to tidal flushing, we set up seven soil treatments (Table 3), with replication

Table 1. Organic Carbon and Total Nitrogen in Natural and Constructed Salt Marshes

		Organic Carbon (%)		Total Nitrogen (mg/kg dry weight)	
Site	Age (yr)	Natural	Constructed	Natural	Constructed
Texas	1	0.3–1.12	0.13	227– 588	95
North Carolina	10–15	0.6–8.6	0.6–1.8	364–1680	322–924
California	4	2.0–2.5	0.1–1.1	1740–2270	870–960

Note: See text for references. Age is the time between marsh construction and sampling.

in each of four blocks, for a total of 28 plots. Tidal flushing began on March 5; a graduate ecology class planted cordgrass (10 pots/treatment plot) on March 21, 1990, and soil salinities, plant numbers, and plant heights were measured periodically from April through September.

Plots of cordgrass growth vs. time (Figure 2) indicate strong responses to treatments with both nitrogen and organic matter added (A + N, S + N), and slow growth without either added (C, R). Statistical analyses of the September biomass (total stem length data) indicate both block and treatment effects (two-way anova, with blocks and treatments as factors, $P < .001$). Block 1 plants without soil amendments grew well, and additions of nutrients had less effect. This block effect is explained by the higher amount of organic matter initially present in the soil in Block 1, which was not known at the time of planting (Table 4). The difference between Block 1 and Blocks 2–4 set up a "natural experiment" and gave the results that would be expected; namely, better growth of cordgrass without further soil amendments and less improvement in growth with addition of nutrients.

The mean growth of cordgrass was highest in plots that had both a nitrogen-rich form of organic matter and additions of inorganic nitrogen (Figure 2). Because of the block effect, treatment replicates were difficult to analyze. When data for Block 1 were omitted, analysis of variance still showed the treatment effect, but the block effect was eliminated. For Blocks 2–4, the highest treatment mean (for A + N) was ~10 times the mean for the lowest (R), and the 95% confidence limits for the two treatments did not overlap. Further analyses are planned to take advantage of the high variability within and between blocks in order to determine additional factors (e.g., soil salinity, elevation, redox, pH) that affect plant growth and vegetative reproduction in this artificial marsh site.

The potential for accelerating the growth of cordgrass by using soil amendments appears to be great. Longer-term evaluation is needed to check for reversals or shifts in responses. As is clear from Figure 2, treatment means did not rank the same at each measurement period; patterns that are established soon after soil amendment are not necessarily sustained as nutrients cycle between organic and inorganic compartments of the ecosystem. It is also possible that changes will

Table 3. Experimental Treatments Established at Marisma de Nación, San Diego Bay, on Feburary 27, 1990

Plot	Treatment
C	Control, no treatment
R	Rototill only, multiple passes
S	Straw (low-nitrogen organic matter), 3 kg/m^2, rototilled into the soil
A	Alfalfa (high-nitrogen organic matter), 3 kg/m^2, rototilled into the soil
N	Inorganic nitrogen fertilizer (ammonium sulfate), 11.2 g nitrogen/m^2, rototilled into the soil
S + N	Straw and nitrogen fertilizer rototilled into the soil
A + N	Alfalfa and nitrogen rototilled into the soil

Plots were 1 x 5 m and were later planted with cordgrass (*Spartina foliosa*).

Table 2. Comparisons of Cordgrass (September 1990) and Extractable Ammonium (July 25, 1990) at the Constructed and Natural Marshes 6 Years after Construction of the Connector Marsh

Sampling stations	Density (stems/m^2)	Total stem length (m/m^2)	Mean of mean heights (cm)	Ext. NH_4, (µg/g dry wt)
Constructed				
NI 1	287 (32)	135 (32)	46	0.86
NI 2	270 (21)	143 (27)	52	1.49
NI 3	273 (24)	115 (14)	42	1.01
NI 4	160 (21)	52 (8)	32	0.96
Natural				
PC 1	283 (9)	184 (4)	65	2.76
PC 2	247 (49)	193 (34)	79	1.80
PC 3	233 (27)	176 (22)	76	1.25

Note: Plant data are means; numbers in parentheses are standard errors. Ammonia data are composite samples for each site. Ext. NH_4 = extractable ammonia.

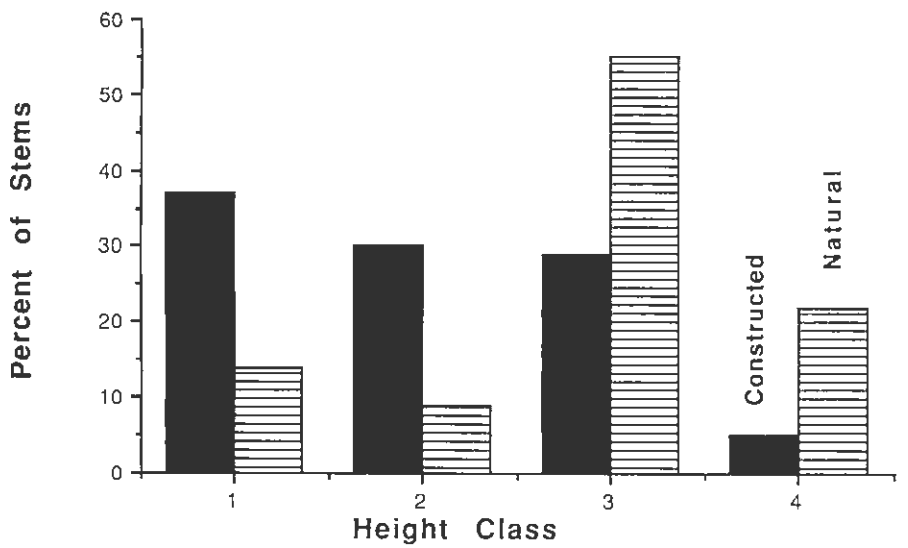

Figure 1. Height frequency distributions for the constructed and natural marshes along San Diego Bay in September 1990.

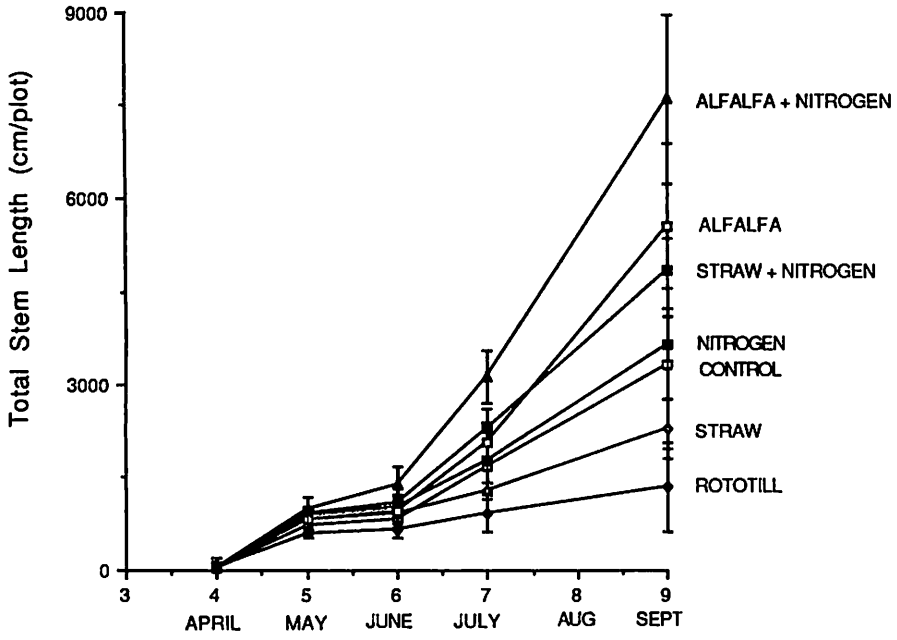

Figure 2. Response of experimental plots at Marisma de Nacion to soil amendments (Table 2). Treatments are described in Table 3. Data are means for n=4 plots; bars = ±1 standard error.

Table 4. Salinity, Amount of Organic Matter, and Texture of Soils at Marisma de Nacion.

	Salinity (Mean of All Plots Measured)							
Date	3/7	3/21	4/2	4/25	5/16	7/11	8/6	9/26
ppt	80	64	62	53	56	51	55	48

	Organic Matter (%) 3/7/90		Texture (%) 2/27/90		
Block	Control	Alfalfa	Sand	Silt	Clay
1	3.8	3.5	64	15	21
2	2.1	4.0	62	15	23
3	2.1	5.9	56	14	30
4	2.1	2.7	68	16	16

Note: ppt = parts per thousand.

Table 5. Extractable Ammonia from the Marisma de Nacion Treatment Plots Collected on 25 July, 1990

	Treatment						
	C	R	S	A	N	S + N	A + N
Means	1.69	2.50	2.91	10.00	8.78	12.00	10.00
Standard error	0.72	1.40	0.77	3.01	3.75	4.99	4.13

Note: Data are means of five replicates (µg/g sediment dry weight). See Table 3 for explanation of treatments.

develop in the consumer communities. Scale insects are a problem in two other constructed wetlands of San Diego Bay, and such herbivores may be stimulated by nutrient-rich vegetation. Whether fertilized plots will be damaged by herbivory may not be known for several years. Thus, although the initial results are promising, the long-term effects of augmenting soils must still be determined, with careful study of the processes that are responsible for the development of ecosystems.

If the current pattern (increased growth of cordgrass after additions of nitrogen-rich organic matter and nitrogen fertilizer) continues through the long term, then it should be possible to accelerate the rate of ecosystem maturation for constructed and restored wetlands by manipulating the organic matter in the sediments.

Cooperating Organizations

California Department of Transportation
San Diego State University graduate student volunteers
U.S. Fish and Wildlife Service, Sweetwater Marsh National Wildlife Refuge

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Zedler, J. B. Restoring salt marshes in southern California. NOAA Symposium on Habitat Restoration: Restoring the Nation's Marine Environment. Washington, D.C., September 25, 1990.

Zedler, J. B., and R. Langis. Functional inequivalency of constructed and natural salt marshes. Annual Meeting of the Society of Wetland Scientists, Breckenridge, Colorado, June 7, 1990.

Testimony

Zedler, J. B. How to achieve "No Net Loss" of California wetlands. Domestic Policy Council, Wetlands Task Force Hearing, Olympia, Washington, September 5, 1990.

Education

California and the Pacific: Marine Sciences for the Public

Moss Landing Marine Laboratories
A/PE-2
Project Initiated: October 1, 1987
Project Completed: September 30, 1990

James T. Harvey

The initial objective of this project was to promote the transfer of information on marine science to the public by using workshops for teachers. In this program, marine scientists would provide current information and relevant educational material that could be used in classroom teaching. This report summarizes the results of an extension of this project; the additional objective was to create teaching material on the ecology of Monterey Bay.

Ten drawings were developed that depict various environments, species, and oceanic processes within Monterey Bay (see, for example, Figure 1). These drawings show some of the major features of Monterey Bay: (1) pelagic area (cetaceans, plankton, pelagic predators, deep sea fishes), (2) kelp forest (kelp frond inhabitants, sea otters/ invertebrates), (3) seabirds, (4) wharf pilings, and (5) mudflats. A geographical drawing shows the locations of major features (e.g., Monterey Submarine Canyon, kelp forests, currents, towns) in the vicinity of Monterey Bay.

These drawings were developed into slides and used for a presentation at the Western Society of Naturalists (Cailliet and Harvey, 1990) and in three presentations for elementary school classes in San Jose and Santa Cruz. These figures will also be used to produce a poster on the ecology of Monterey Bay and will be used in an interactive video produced at Hollister Elementary School by Thomas Keating. This interactive computer video allows the student to select certain aspects of the marine environment in Monterey Bay for text, graphical, or video display. A map of Monterey Bay begins the session, and the student uses a computer mouse to select portions of the map for detailed investigation. For instance, by selecting a fish on the screen in the middle of the bay, a short video (on CD ROM) about pelagic fishes is displayed. The drawings serve as introductory material with accompanying text. The drawings are intended to depict their topic generally, by showing abundant or typical species. The artwork, therefore, summarizes the subject before the detailed video portion begins. Eventually, the drawings on the screen will be blocked so the pointer can be used to select certain parts of the drawing for investigation of further subjects. For example, the drawing of the wharf piling might appear after the user selects the marina environment from the map. After the drawing of the piling appears, selection of the barnacles on the piling will elicit a short video on feeding of barnacles.

The interactive video will allow students to explore the marine environment at their own pace and at a complexity related to their interest and capabilities. The drawings will serve as the first introduction to these subjects and with accompanying text will provide information on species names, food habits, distribution, behaviors, and unique characteristics. These drawings, and their use in posters, computer-assisted interactive videos, and visual aids for seminars, are consistent with the objective of this project to provide educational material on marine science to the public. Visual displays of marine science are one of the best and most esthetic ways of conveying such information. These drawings will probably be used for a variety of other functions besides those already planned.

References

Cailliet, G. M., and J. T. Harvey. 1990. Assemblages of pelagic nekton (squid, fishes, birds, and mammals) and their trophic interactions in Monterey Bay. Paper presented at the Western Society of Naturalists, Monterey, California.

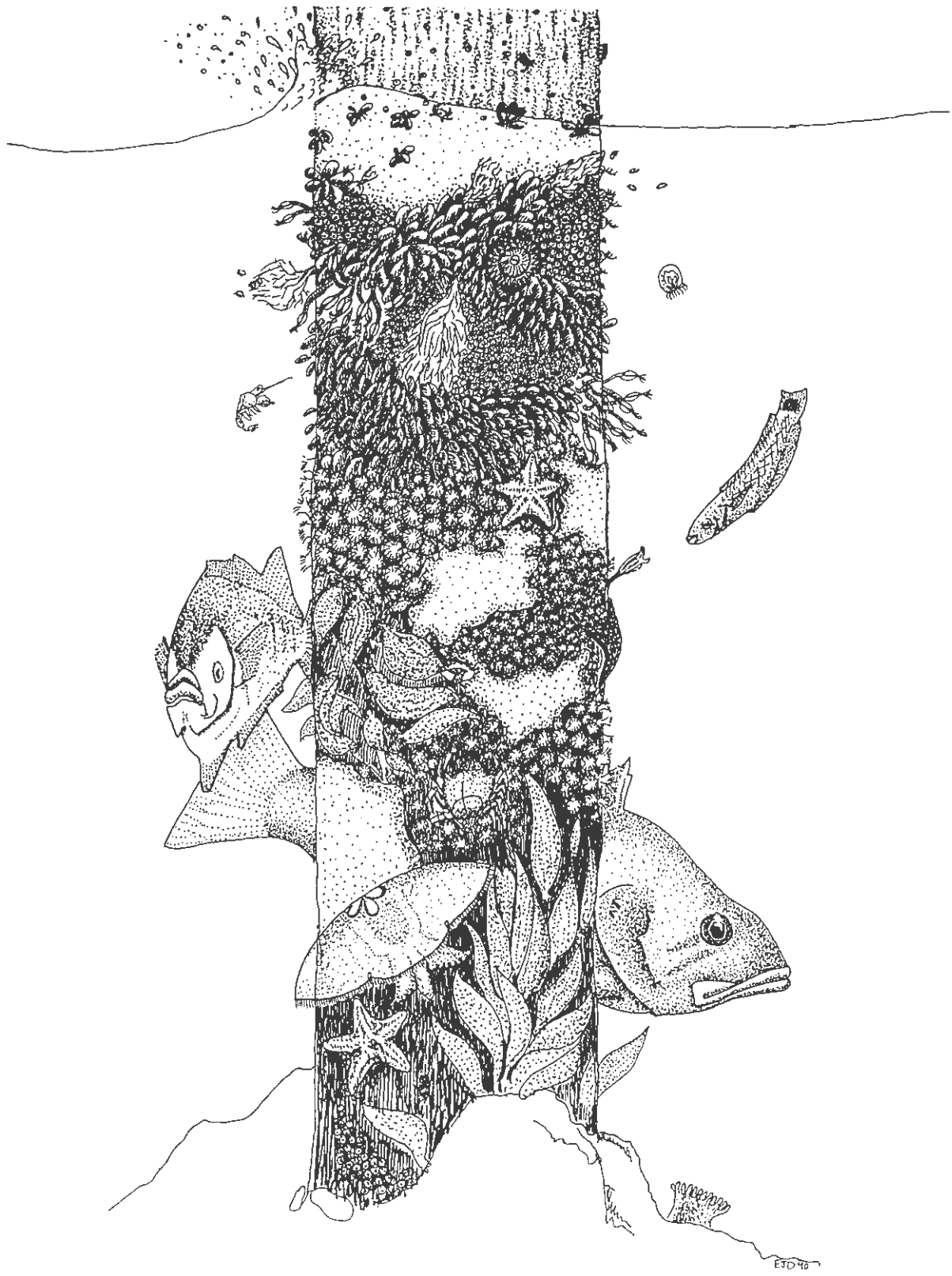

Figure 1. Wharf pilings offer vertical spaces for many invertebrate organisms. Because of the changes in water level associated with the tides, different organisms are found along the pilings, according to the organisms' tolerance to waves, desiccation, and light. Going down along this piling are small algae, mussels, barnacles, a starfish, tunicates, a crab, and larger brown algae. In the water surrounding the piling are fishes (surfperch, rockfish, and a senorita) and jellyfish.

Continuing Projects

Sea Grant Extension Program

The work of the California Sea Grant Extension Program (SGEP) is organized at present into four major program areas: Marine Fisheries, Seafood Technology, Coastal Resources, and Aquaculture.

MARINE FISHERIES

Marine fisheries remains the most extensive program area within the Sea Grant Extension Program. SGEP staff have divided the marine fisheries program into five subprogram areas (1) Fisheries Efficiency and Safety; (2) Fisheries Utilization and Management; (3) Fisheries Enhancement; (4) Fisheries Education; and (5) Fishing Gear Technology.

Fisheries Efficiency and Safety

Improving fishermen's safety practices and energy efficiency is the major emphasis of this subprogram.

Selected activities and accomplishments for 1989-90 in the area of energy efficiency are as follows: We completed a detailed evaluation of the energy loan program. It showed that fishermen saved an average of 17.1% on their fuel bills. The $668,000 of loans saved 723,800 gallons of fuel. We recommended to the California Energy Extension Service that the loan program be expanded. Fred Jurick worked with Eureka area fishermen and gear manufacturers to test kort nozzles and energy-efficient net designs. Several marine advisors distributed sea-surface temperature charts to help fishermen reduce search time. Descriptions of the project were published in *Fishermen's News* and the *Marine Fisheries Engineering and Technology Review.*

As part of our fishing safety project, a marine safety manual in Vietnamese was completed and distributed. This manual enhances the safety video produced in 1988-89. Marine advisors also participated in a national safety survey, and they publicized the availability of safety grants by the Secretary of Environmental Affairs. Leigh Johnson documented that nine local fishermen received $24,131 in grants directly because of her efforts. Jim Waldvogel obtained funding for a weather fax to help fishermen predict sea conditions. Several marine advisors held safety workshops and worked closely with industry and the Coast Guard on new safety regulations.

Utilization and Management

This subprogram was established to provide technical and research information on newly developed and established fisheries.

During the past two years, the new hagfish fishery has attracted up to 35 vessels. Connie Ryan and Sus Kato (NMFS) completed their study on fecundity, size at first reproduction, and size frequency of Pacific hagfish landed in San Francisco. Ed Melvin received a grant from NMFS to study hagfish skin quality. He collaborated with the Monterey Bay Aquarium Research Institute (MBARI) to study escapement as a function of hole size using a submersible video camera. Melvin also collaborated with Greg Cailliet in a study of the life history of hagfish.

Onboard refrigeration and test marketing projects were completed and the results disseminated through industry workshops, newsletters, and reports. Funding ($62,000) was obtained from the California Competitive Technology Program to commercialize refrigeration and chilling of anglers' catches onboard charter/party boats. A paper on the project was accepted in *Marine Fisheries Review.* Fred Jurick completed a leaflet on Humboldt County recreational fishing opportunities.

Chris Dewees and Ed Ueber (NMFS) convened a National Workshop on Bycatch Issues and edited the proceedings. Chris Dewees completed an invited review paper on individual transferable quotas (ITQs) for *Society and Natural Resources.* He also continued to work with the sea urchin industry and Department of Fish and Game to develop fishery management schemes.

Fisheries Enhancement

Need exists to enhance fisheries through habitat-improvement projects and improved fishery-management techniques, to train public enhancement groups in new rearing and habitat techniques, and to improve communication among public enhancement groups.

Jim Waldvogel edited and distributed 300 copies of the *Proceedings of the Eighth California Salmon, Steelhead and Trout Enhancement Conference.* Bruce Wyatt carried out a stream-enhancement project with Choices for Change, an agency working with families at risk. Ed Melvin completed the adult steelhead population sampling program on Scott Creek. Melvin's salmonid thyroxine sampling program, conducted in cooperation with Howard Bern's Sea Grant project, was completed.

The spawning estimate for fall chinook salmon on Mill Creek (Smith River) was completed for 1989.

Leigh Johnson held several planning sessions with kelp industry, fishery, and agency representatives to plan a sea urchin and kelp enhancement project.

Marine Fisheries Education

Jim Waldvogel trained 12 Del Norte County teachers on the use of classroom salmonid egg incubators, and he determined that over 900 students were reached by his efforts. Connie Ryan taught a three-unit class at San Francisco State on the Salmon and Trout Education Program (STEP) to 26

teachers. All SGEP staff supplied marine education assistance to teachers and 4-H programs.

Funding was received for *California's Living Marine Resources* in August 1990. Format, content, and 75 contributing authors were determined.

Fishing Gear Technology

The fishing gear technology subprogram area involves developing techniques for supplemental fisheries, improving new gear technology, and reducing gear conflicts.

Dewees, Richards, and Price reviewed dozens of proposals for the Environmental Affairs Agency. Fred Jurick worked with net manufacturers to develop acceptable fish separator trawls. He also was named to the National Seafood Safety Vessel Certification Steering Committee.

Initial field studies of the sheep crab fishery were completed by Carrie Culver, and funding was obtained from the Local Marine Fisheries Impact Program to expand the study with the use of a remotely operated vehicle (ROV).

SEAFOOD TECHNOLOGY

Seafood technology research and educational needs were identified through interactions with seafood industry associations, the Sea Grant Seafood Industry Advisory Committee, Cooperative Extension Marine Advisors, agencies, societies, and through individual contacts with the seafood industry.

On-Board Handling and Quality Control

During 1989-90, the Seafood Technology Specialist Bob Price and Marine Advisor Ed Melvin completed two manuscripts based on research from this project, and drafted a pamphlet for commercial fishermen.

Seafood Quality Improvement

Cooperative Extension workshops and short courses on canning technology, food processing sanitation, statistical quality control, and freezing technology provided seafood industry personnel with needed and useful information.

Fred Jurick participated in a regional vessel seafood inspection workshop sponsored by the National Marine Fisheries Service and the National Fisheries Institute. He was appointed to the National Seafood Safety Vessel Certification Steering Committee.

Safe Handling of Seafood

Bob Price and colleagues from the U.S. Department of Agriculture and Cooperative Extension are conducting a project to evaluate the effectiveness of the mass media in increasing consumer awareness of risks associated with seafood usage as well as practices that will reduce risk.

Extension personnel surveyed Sacramento seafood retail markets to determine potential safety problems.

Educational leaflets for retailers and consumers on specific seafood safety problems and on proper handling techniques to prevent problems were produced.

Workshops were held for seafood retailers, county health inspectors, and food editors on proper retail seafood handling. Fourteen publications for the industry provided additional information on safe handling.

Seafood Safety

Leigh Taylor Johnson and Robert Price are participating in a study of media reports of seafood contamination.

Johnson organized a "Seafood Issues" session for the San Diego County Cooperative Extension Conference on "Pesticides and Contaminants in Our Food and Environment." A *San Diego Union* reporter produced a balanced article entitled "Less Seafood Eaten: Toxic Scare Said a Reason," which reached an extensive audience.

A publication on contaminants in fishes was published, and another on paralytic shellfish poisoning was revised.

Seafood Solid Waste Management

The goal of this project led by Bruce Wyatt is to inform farmers, fertilizer suppliers, farm advisors, and organic growers and gardeners of the uses of seafood processing waste.

Results of research on utilization of seafood processing waste were presented at a regional and an international conference. A publication based on this research, *Use of Marine By-Products on Agricultural Crops*, is in press. Publications on *Making Fish Compost at Home* and *Fish Emulsion: How to Use It on Your Plants* were completed.

Conservation of Seafood Processing Water

Project Leaders Bob Price and Jim Waldvogel are initiating a project to improve water-use efficiency of shrimp processing machines and sea urchin and groundfish filleting operations in coastal communities. They also seek to determine methods to direct high-quality waste water into other seafood processing operations.

Packaging Materials for Home Frozen Storage of Tuna

Leigh Taylor Johnson analyzed results of sensory evaluations conducted after tuna was held in frozen storage under various treatments.

Food Professional Training

Advisor John Richards and an Extension Home Economist conducted a workshop on seafoods for Master Food Preservers in San Luis Obispo.

COASTAL RESOURCES

Many of California's most valued resources and most of its population are found within the coastal zone.

Marine Ecosystem Management

During 1989-90, Marine Advisor Ed Melvin and Postgraduate Research Assistant Michelle Hornberger reviewed the Draft Environmental Impact Statement and Management Plan for the

Monterey Bay Sanctuary.

Bruce Wyatt cooperated in planning and producing the proceedings of the 1990 State of Tomales Bay Conference.

Leigh Johnson chaired the San Diego Bay Symposium.

Over $17 million is expended annually on marine pollution monitoring in southern California. The Sea Grant Extension Program will cooperate in a project to coordinate objectives, methods, and data management for marine monitoring and pollution research programs.

The overall objectives of a project on marine debris are to improve the ability of vessel and marine terminal operators to comply with new U.S. laws and help reduce marine plastic pollution.

In 1989-90, Marine Advisor Fred Jurick played a key role in assisting regional mooring facilities, fish receiving points, fishermen, and recreational boaters in meeting marine debris requirements.

Multiple Use of Coastal Resources

Multiple use of coastal resources presents significant social, technical, and economic challenges.

The "California Coastal Waterfront Managers' Survey" published by Advisors Leigh Johnson and Connie Ryan reported numerous topics for which waterfront managers need research and education programs.

Fred Jurick cooperated on a Noyo Port District study team organized by Oregon State University Sea Grant. He also cooperated with the Humboldt Bay Fisheries Association on waterfront related projects.

Bruce Wyatt organized a project to propose changes in water releases at Coyote Dam on the Russian River. The resulting proposal was sent to the California State Water Resources Control Board.

Jim Waldvogel coordinated and reviewed the completion of the "Del Norte County, Northern California Coastal Fish Habitat and Fishing Areas" overlay maps. The project was completed by the Coastal Resources Center under contract to Del Norte County. The map overlays were developed to coordinate future fishery needs and identify potential OCS/fisheries conflicts (seismic surveys).

Fred Jurick served on the Humboldt County Outer Continental Shelf Oil Advisory Committee, which set priorities and planned mitigation projects utilizing existing funds.

The "Oil & Gas Project Newsletter" and an educational videotape on seismic survey/fisheries conflict resolution were continued by John Richards. He also presented a paper on offshore oil and fishing industry communications and conflict resolution methods at the Minerals Management Service's Pacific Outer Continental Shelf Regional 5th Information Transfer Meeting (ITM) held in Santa Barbara.

During 1989-90, marine advisors presented information through public schools, community events, teacher training sessions, newsletters, professional associations, classroom projects, field days, and environmental education centers.

AQUACULTURE

Commercial shellfish production, primarily oysters, mussels, and abalone and hatchery production of salmon, sturgeon, and striped bass for fisheries mitigation and enhancement are major contributors to the state's marine aquaculture production.

Aquaculture Public Service

Leigh Johnson assisted thirty-eight public service callers with aquaculture questions on fish, invertebrates, and aquatic plants. Johnson also advised planners of the 1991 California Farm conference on aquaculture.

John Richards wrote a review of the potential for both marine subtidal leasing for shellfish mariculture and land-based aquaculture and its relationship to traditional agriculture. The potential for water-savings using land-based marine systems was also presented. The review was used in the Santa Barbara County Planning Commission's decision-making process for allowing land-based abalone hatchery and grow-out facilities to operate on coastal agricultural lands.

Richards also hosted a nine-member Chinese delegation of marine aquaculturists from Dalian, northern China, and provided opportunities for the exchange of research and technical information with biotechnology researchers at UCSB and shellfish growers in Santa Barbara.

Richards also was elected to the National Shellfish Association Board of Directors for 1990-91.

Water Quality and Shellfish Sanitation

Bruce Wyatt held meetings with the Tomales Bay Shellfish Growers to inform them of agency activities related to aquaculture activities. These meetings have led to agency adjustments in research activities in Tomales Bay to better define the dairy waste problem and its effects on shellfish growers. Both upstream and in-bay water-quality sampling has been increased.

Leigh Johnson has cooperated with Seafarms West mussel farm in San Diego County's Aqua Hedionda Lagoon, which has been threatened by elevated fecal coliform levels for two years.

John Richards served as a member of the Technical Advisory Committee (TAC) for the Central Coast Regional Water Quality Control Board's project entitled "Non-Point Source Evaluation and Cleanup Strategy for Shellfish Contamination in the Santa Barbara Channel."

New Aquaculture Species Technology Development

The overall objectives of this project are to identify and encourage research on new aquaculture species, improve culture techniques, and to assist growers in testing these new species and techniques for commercial production.

John Richards contacted mussel

growers in Washington State and obtained promising low-cost substrates for mussel seed settling experiments. He provided literature on bivalve hatchery and remote settling methodology to Kamelche Sea Farms, Shelton, Washington, to assist with the development of a regional hatchery mussel seed grow-out project submitted for 1991 funding to the National Coastal Resources Institute (NCRI).

Marine Fisheries Specialist Christopher Dewees chaired the research sub-committee of the CDFG Director's Sea Urchin Advisory Committee. He reviewed proposals and contracts for funding sea urchin culture and enhancement research.

Bruce Wyatt initiated research in Tomales Bay to determine market preferences for blue mussels related to season and size.

John Richards cooperated with a San Diego State University sea urchin researcher to develop and expand his statewide sea urchin settlement research in the Santa Barbara Channel. John Richards and Carrie Culver also completed the review of an independent-studies project on mussel seed.

Offshore Mariculture and Commercial Fishing Operations

Offshore mariculture has increased off Santa Barbara County, and some of the mariculture operations interfere with commercial fishing operations.

John Richards met with California Department of Fish and Game aquaculture coordinators to review potential conflict resolution methods and to plan a second joint meeting to review operations of both industries and improve local communication efforts.

Cooperating Organizations

ABB Environmental Services, Inc.
Ab Lab
Abalone Farms, Inc.
Abalone International
Adopt-a-Beach
Alaska Commercial Fisheries Entry Commission
Alaska Sea Grant College Program
American Fisheries Society
American Fisheries Society, Humboldt Chapter
American Tunaboat Association
Aquaculture Digest
Assemblywoman Lucy Killea's Office
Assemblywoman Sunny Mojonnier's Office
Association of Monterey Bay Governments
Atlantic Offshore Fishermen's Association
Audubon Society
Battelle Memorial Institute
Bay Bottom Beds
Benech Biological, Inc.
Big Lagoon Rancheria
Blue Lake Rancheria
Bodega Bay Fisheries Marketing Association
Bordynsky (Joe), C.P.A.
C&N Fisheries
California Abalone Association
California Academy of Sciences
California Aquaculture Association
California Association of Harbor Masters and Port Captains
California Beef Council
California Certified Organic Farmers
California Coastal Commission
California Coastal Operators Group
California Department of Boating and Waterways
California Department of Commerce, Office of Competitive Technology
California Department of Corrections
California Department of Fish and Game
California Department of Forestry
California Department of Health Services
California Department of Parks and Recreation
California Energy Extension Service
California Farm Bureau
California Fish Growers
California Fisheries and Seafood Institute
California Gillnetter's Association
California Marine Mammal Center
California Marine Parks and Harbors Association
California Maritime Academy
California Office of Environmental Affairs
California Office of Planning and Research
California Salmon, Steelhead, and Trout Restoration Federation
California Spray Dry
California State Coastal Conservancy
California State Lands Commission
California State University, Chico
California State University, Hayward
California State Water Resources Control Board
California Trout
California Urchin Divers Association
Call of the Sea
Canadian Department of Fisheries and Oceans, British Columbia
Canel Alliance
Carmel River Steelhead Association
Castle Rock Seafood, Inc.
Channel Island National Marine Sanctuary
Chesapeake Fish Company
Choices for Change
Chetco STEP, Inc.
Circuit Rider Productions
Cloudburst Fishing Company
Coast Oyster Company
Coastal Fisheries Foundation
Coastal Resources Foundation
Coastal Resources Center
College of the Redwoods
Commercial Fishermen of Santa Barbara, Inc.
Commercial Fishermen's Wives of Humboldt
Congressman Doug Bosco's Office
Congressman Jim Bates' Office
Crescent City Harbor District
Crescent City Parks and Recreation
Cuesta College
The Cultured Abalone
Curry County Commissioners
Curry County Fishermen's Association
Dana Wharf Sportfishing
Danish Institute of Fisheries Technology
Del Ackerlund Farms, Valley, Nebraska
Del Norte County Board of Supervisors
Del Norte Fishermen's Marketing Association
Devoe Paint Company
ECOMAR, Inc.
EG and G Oceanographic Services
ERC Environmental and Energy Services
El Granada Elementary School
Elk Valley Rancheria
Elkhorn Slough Foundation
Elkhorn Slough National Estuarine Research Reserve
Environmental Health Coalition
Environmental Satellite Service
Eureka Fisheries, Inc.
Eureka Times-Standard
F/V *Abrigo*
F/V *Apollo*
F/V *Azalea*
F/V *Cindy J.*
F/V *Excalibur*
F/V *Flying Fish*
F/V *Fred Holmes*
F/V *Freelance*
F/V *Ginnie C II*
F/V *Gus D.*
F/V *Hecate*
F/V *Jenna Lee*
F/V *Mr. Bill*
F/V *New LoAn*
F/V *Pt. Loma*
F/V *Salty Lady*

F/V *Seastar*
F/V *Steelfin II*
F/V *Webfoot*
"Fish Phone"
Fisheries Protection Institute
Fisheries and Oil Industries Joint Committee
Fisheries and Oil Industries Liaison Office
Fishermen's Cooperative Association
Fishermen's Marketing Association, Inc.
Fishermen's Union—I.C.W.U. Local 33
Florida Sea Grant College Program
Friends of Lobos Creek
Golden Gate Fishermen's Association
Golden Gate National Recreation Area
Gray, Cary, Ames and Frye
Great Barrier Reef Authority
Gregorio Aquatech
Gulf of the Farallones National Marine Sanctuary
Half Moon Bay Fishermen's Association
Hawaii Sea Grant College Program
Hog Island Oyster Company
Hopkins Marine Station
Hoopa Valley Reservation
Howorth & Associates, Santa Barbara
Humboldt Bay Fisheries Association
Humboldt Bay Harbor, Recreation, and Conservation District
Humboldt County Board of Supervisors
Humboldt County Office of Education
Humboldt County Planning Department
Humboldt Fish Action Council
Humboldt Fishermen's Marketing Association
Humboldt Senior Resource Center
Humboldt State University
IKA Venture Corporation, Ltd., Auckland, New Zealand
Instacool Inc. of North America, Rancho Cordova
International Marina Institute
J.J. Camillo Seafood Company
Joint Committee on Fisheries and Aquaculture
KGO-TV, San Francisco
KVP Research
Kamilche Sea Farms, Washington
Kelco
Klamath Management Council
LMR Resources, Inc.
The Log
Long Marine Laboratory
Los Angeles County Department of Beaches and Harbors
Louisiana Sea Grant College Program
Mar-Cal Seafood
Marin Rod and Gun Club
Marin County Board of Supervisors
Marin Wildlife and Fisheries Advisory Committee
Marine Associations Council of California
Massachusetts Division of Marine Fisheries
Massachusetts Institute of Technology Sea Grant College Program
Mediation Institute
Mendocino County Board of Supervisors
Mendocino County Department of Environmental Health
Meredith Fish Company
Miller-Rellim Redwood Company
Monterey Bay Anadromous Fish Advisory Committee
Monterey Bay Aquarium
Monterey Bay Aquarium Research Institute
Monterey Bay Salmon and Trout Project
Monterey County Health Department
Monterey Harbor, City of Monterey
Bob Morrel Enterprises, Inc.
Morro Bay Commercial Fishermen's Association
Morro Bay Harbor Department
Moss Landing Commercial Fishermen's Association
Moss Landing Harbor District
Moss Landing Marine Laboratories
Moss Landing Marine Supply
NOYO Women for Fisheries
National Coastal Resources Institute
National Fisheries Institute
National Fisherman Magazine
National Marine Educator's Association
National Marine Fisheries Service
National Marine Manufacturers Association
National Shellfisheries Association
National Weather Service
Nationwide Marketing
The Nature Conservancy
Naval Ocean Systems Center
Navy Post Graduate School
New Growth Forestry Services
New Jersey Sea Grant College Program
New York Sea Grant College Program
New Zealand Federation of Commercial Fishermen
New Zealand Ministry of Agriculture and Fisheries
Noble Associates
Nor Cal Truck Specialties
North Carolina Sea Grant College Program
North Coast View Magazine
North Pacific Fisheries Management Council
Northern California Federation of Fly Fishers
Noyo Port District
Oceanic Society
Oceanside City Harbor District
Office of Coastal Resource Management
Orange County Marine Institute
Orange County Register
Oregon Department of Fish and Wildlife
Oregon Sea Grant College Program
Oregon Southcoast Sportfishermen's Association
Pacific Choice Seafoods
Pacific Coast Congress of Harbor Masters and Port Managers
Pacific Coast Federation of Fishermen's Associations, Inc.
Pacific Coast Fishermen's Wives Coalition
Pacific Coast Guides Association
Pacific Coast Oyster Growers Association
Pacific Edge, Inc., Costa Mesa
Pacific Fishery Management Council
Pacific Mariculture Inc.
Pacific Marine Fisheries Commission
Pacific Trawl Company
Palo Alto Times-Tribune
Pillar Point Harbor
Point Reyes Oyster Co.
Point St. George Seafoods, Inc.
Port of Brookings
Port of Gold Beach
Port of Port Orford
Port of San Diego
Port San Luis Commercial Fishermen and Boat Owners Association
Presidential Outer Continental Shelf Leasing and Development Task Force
Provincial Fisheries Department, British Columbia
Puget Sound Water Quality Authority
Quality Refrigeration Co.
Queensland Fisheries Management Authority
Radio KCRE
Radio KFLI
Radio KPOD
Radio KURY
Redondo Beach King Harbor District
Redway Elementary School
Redwood Community Action Association
Rogue River Guides Association
Rural Human Services
Salmon Trollers Marketing Association of Fort Bragg
Salmon Unlimited
San Diego County Board of Supervisors
San Diego County Department of Agriculture
San Diego County Department of Health Services
San Diego County Public Health Department
San Diego Dockmasters Group
San Diego Regional Water Quality Control Board
San Diego Cowbelles
San Diego Fishermen's Association
San Diego Log
San Diego Marine Trade Association
San Diego Oceans Foundation

San Diego Seafood Association
San Diego Sportfishing Association
San Diego State University
San Diego Union
San Francisco Bay Fishermen's Association
San Diego Port Tenants Association
San Diego Seafood Association
San Francisco Unified School District
San Jose State University
San Lorenzo River Steelheaders
San Luis Obispo County Planning Department
San Mateo County Department of Parks and Recreation
San Mateo County Department of Education
Santa Barbara City Planning Department
Santa Barbara Commercial Fishermen
Santa Barbara County Board of Supervisors
Santa Barbara County Fish and Game Commission
Santa Barbara County Resource Management Department
Santa Barbara Harbor Department
Santa Barbara Museum of Natural History
Santa Barbara Sea Center
Santa Cruz Commercial Fishermen's Association
Santa Cruz County Planning Department
Santa Cruz Port District
Santa Monica Bay Restoration Project
Science Applications International
Scow Enterprises, Fremont, Minnesota
Scripps Aquarium/Museum
Sea Farms West
Sea Products, Inc.
Seafood Specialties
Society for Applied Anthropology
Sonoma County Grape Growers
Sonoma Fish and Game Advisory Committee
Southern California Coastal Water Research Project
Southern California Lobstermen's Association
Southern California Marine Association
Southland Farmer's Market Association
Southwest Marine Educators Association
Sportfishing Association of California
Spud Point Marina Bodega Bar
Squid Machine Corp., Watsonville
State Fish Company
Tomales Bay Oyster Company
Tomales Bay Oyster Growers Association
Tomales Bay Shellfish Growers Association
Town Dock Seafood, Galilee, Rhode Island
Trans National Agronomic, Grand Rapids, Michigan
Trinidad Fishermen's Marketing Association
United Anglers of California
United States Tunaboat Association
U.S. Army Corps of Engineers
U.S. Bureau of Indian Affairs
U.S. Coast Guard
U.S. Department of Energy
U.S. Department of Interior
U.S. Department of Interior Minerals Management Service
U.S. Environmental Protection Agency
U.S. Fish and Wildlife Service
U.S. Food and Drug Administration
U.S. Naval Base, San Diego
U.S. Soil Conservation Service
United Anglers of California
United Anglers, Inc.
University of California, Davis, Bodega Marine Laboratory
University of California, Berkeley Department of Naval Architecture
University of California, Santa Barbara
University of California, Santa Cruz, Long Marine Laboratory
University of Minnesota Cooperative Extension
University of Rhode Island, Marine Advisory Service
University of Rhode Island, Master Gardner Coordinator
University of Southern California Sea Grant Program
University of Washington, Manchester Laboratory
Ventura Harbor Department
Vietnamese Fishermen's Association of America
Vietnamese Pacific Fishermen's Association
W. R. Merry Seafood, Inc.
Warren Webber Farms
Washington Department of Ecology
Washington Department of Fisheries
Washington Department of Game
Washington Sea Grant College Program
Washington State Legislature
Waterfront Press, Seattle
West Coast Fisheries Development Foundation
Western Association for the Valuation of Ecosystems (WAVE)
Western Fishboat Owners Association
Western Oil and Gas Association
Westlog, Inc.
Wisconsin Department of Natural Resources
Women's Fisheries Network
Yurok Transition Team

Communications

The Communications Office of the California Sea Grant College plays an essential role in disseminating information about the activities and accomplishments of the program and in promoting communication among a variety of audiences involved in marine resource management, conservation, and development.

Located at the program's administrative headquarters at UC San Diego, the Communications Office has these major objectives:

1. To inform a wide spectrum of audiences about the mission and activities of the state and national Sea Grant programs;
2. To inform public, industry, scientific, legislative, and other audiences about findings arising from Sea Grant-sponsored research;
3. To educate a wide spectrum of audiences about state, national, and international marine-resource issues;
4. To assist and support the information dissemination activities of program management.

Background

The California Sea Grant College is the largest in the national network. The state it serves has 15 coastal counties stretched along a thousand-mile coastline. Eighty percent of California's population, or some 24 million people, are estimated to live within 30 miles of this coast, and the population continues to grow rapidly. Given the concentration of people along the coast and the wealth of resources in the Pacific Ocean, marine-related issues are extremely important within the state. These issues are reflected in the research, education, and advisory activities of the program, and range from the health and viability of California's fisheries to the vulnerability of the coast to erosion and the effects of offshore oil development.

Publications Rationale

Because the potential of Sea Grant research and other activities is not met unless the results generated get into appropriate hands, the work of our principal investigators is reported at different levels for different audiences. Most of our efforts are directed to reaching leaders in the legislature, academia, government agencies, and industry.

Three publications form the foundation of our publications efforts. The first is an annual *Program Directory* of currently funded projects. This publication provides a general program overview plus a guide to current Sea Grant-sponsored work throughout the state.

A second publication which we consider fundamental is our *Summary*, perhaps our major public information product. Written for the educated layman, the *Summary* allows us to report noteworthy accomplishments in all of our spheres of activity and to develop a number of themes that set program activities in a different or larger context.

A third core publication is the *Biennial Report of Completed Projects*, in which each principal investigator reports his or her progress in language appropriate for peers. It forms an essential historical record of program accomplishments, including publications and results, and thus represents an important document in terms of both program accountability and dissemination of scientific and technical results.

Additional publications reflect areas of special interest or emphasis within the program. In 1989, an 80-page report reviewed highlights of California Sea Grant's 20-year history and served as the recertification report; it was titled *Sea Grant in California: Twenty Years of Achievement*. Publications produced in the 1988-90 period are listed at the end of this report.

Dissemination

It is the policy of California Sea Grant to encourage researchers to publish their results in professional journals. The Publications Office attempts to monitor the publications activity of our researchers as one important measure of program productivity and to disseminate all published materials to appropriate parties.

In addition to our standard distribution procedures, each title is added to a widely distributed publications list (issued twice yearly by the Publications Office) as well as to *Sea Grant Abstracts*, which is distributed nationally.

In 1989-90, the Information Specialist distributed reprints of 60 journal articles and papers from published conference proceedings. In addition, she distributed publications in the California Sea Grant series (produced by this department) and miscellaneous publications in a number of categories for a total of 104 different items, or 6,083 pieces. Addition of publication announcements, press releases, and awards announcements brought the number of pieces distributed to 25,452.

The Information Specialist not only handles initial distribution of publications, but also maintains files of reprints and books from which to fill both specific and general requests for information. In 1987-88, there were 900 "unsolicited" requests for information or publications (i.e., not directly generated by our own publications announcements), bringing the total number of pieces distributed to 28,029.

Public Information and Special Projects

The Communications Office is responsible for media relations and public information activities, such as issuing press releases. It also produces a number of miscellaneous products on an annual basis. These include portions of the institutional proposal, brochures, certificates and plaques, acknowledgement and reprint guidelines, and the Call for Annual Reports. The Office also provides assistance to the Program Manager on special projects as requested.

Sea Grant Reference Series

Amidei, R. 1989. *Sea Grant in California: Twenty Years of Achievement.* Published as the principal document of the Recertification of the California Sea Grant College on May 8-11, 1989. No. R-CSGCP-026. 80 pages, 17 photographs, 9 figures.

California Sea Grant College. *California Sea Grant Program Directory 1989–90.* No. R-CSGCP-027. 28 pages, 6 photographs.

California Sea Grant College. 1990. *California Sea Grant Biennial Report of Completed Projects, 1986–88.* No. R-CSGCP-028. 155 pages, 54 figures, 21 tables.

California Sea Grant College. 1990. *California Sea Grant Program Directory 1990–91.* No. R-CSGCP-029. 29 pages, 3 photographs.

Sea Grant Technical Series

Abbott, I. A. Editor. 1988. *Taxonomy of Economic Seaweeds with reference to some Pacific and Caribbean species,* Volume II. Results of an international workshop sponsored by the California Sea Grant College and the Institute of Oceanology of the Academia Sinica of the People's Republic of China in cooperation with the Pacific Sea Grant College Programs of Alaska, Hawaii, Oregon, and Washington and hosted by the Institute of Oceanology in Qingdao, September 22-25, 1986. No. T-CSGCP-018. 265 pages, 268 figures, 4 tables.

Dewees, C. M., and E. Ueber, Eds. 1990. *Effects of Different Fishery Management Schemes on Bycatch, Joint Catch, and Discards.* Summary of a national workshop sponsored by the California Sea Grant College and the National Marine Fisheries Service, held at San Francisco, California, January 29–31, 1990. No. T-CSGCP-019. 55 pages.

Pacific Estuarine Research Laboratory. 1990. *A Manual for Assessing Restored and Natural Coastal Wetlands: With Examples from Southern California.* No. T-CSGCP-021. 105 pages, 39 tables, 33 figures.

Working Paper Series

Cicin-Sain, B. 1990. *California and Ocean Management: Problems and Opportunities.* No. P-T-49. 39 pages.

California Sea Grant College. 1990. *Preservation of Ageing Marine Structures: Conference Notes from the California Sea Grant Symposium.* No. P-T-50. 36 pages.

Lima, J. T. 1990. *Ocean and Coastal Management; The Role and Activities of California Government in Spring 1988.* No. P-T-52. 45 pages.

McGinnis, M. V. 1990. *The Multiple Uses of the Coastal Zone and Ocean Offshore California.* No. P-T-51. 23 pages.

Education

The commitment of California Sea Grant to education and training activities in the marine sciences is evident in the projects it supports for students at all levels, as well as for the general public.

The Trainee Program

Research projects supported by California Sea Grant generally include at least one graduate student trainee. During their training, students work alongside university scientists and engineers in demanding and stimulating research environments, while working on or completing graduate degrees. These young scientists and engineers will be responsible for maintaining the high quality of U.S. marine research in the future. In 1989-90, 67 Sea Grant trainees conducted marine research with project leaders at California universities and colleges.

Isaacs Scholarship

The eighth John D. Isaacs Memorial Sea Grant Scholarship was awarded in 1989 to Michelle Brand of El Cerrito High School for her research on mussels and water pollution.

The ninth John D. Isaacs Memorial Sea Grant Scholarship was awarded in 1990 to Elizabeth Springer, a graduate of Patrick Henry High School, for her research on salt stress and plant growth. The $10,000 award, allocated over a four-year period, recognizes the research excellence of California high school seniors and encourages students to continue their marine education at California colleges and universities. Springer's study was selected from other marine-related projects at the California State Science Fair. She is presently a freshman at Stanford University.

California Sea Grant State Fellowship Program

California Sea Grant's state fellowship program provides a unique educational opportunity for graduate students who are interested in both marine resources and the policy decisions that affect those resources. The program matches highly motivated and qualified graduate students with "hosts" in the California state government or in state agencies for a nine-month paid fellowship. In 1989, Robert J. Wilder, a Ph.D. candidate at the University of California, Santa Barbara, was the Sea Grant state fellow. Wilder was a fellow in the office of Assemblyman Dan Hauser, vice chairperson of the Joint Committee on Fisheries and Aquaculture. In 1990-91, there were two fellows: Julie A. Reynolds, of Claremont College, has been working at the Channel Islands National Marine Sanctuary; and Bruce Wulkan of the University of Washington has been working with the California State Office of the Secretary of Environmental Affairs.

Graduate Research Fellowship Program

In 1990 an experimental program was initiated to support thesis research in marine sciences. The purposes of the program are (1) to provide support for meritorious independent graduate student research and (2) to recognize in an appropriate way the independent contributions of students. In 1990, six students received the award: Kevin Lafferty (University of California, Santa Barbara), Erik V. Thuesen (University of California, Santa Barbara), Blaise J. Eitner (University of California, Los Angeles), Peggy Fong (San Diego State University), Charles Lester (University of California, Santa Barbara), and Melissa Gibbs (San Jose State University).

Appendices

Officials and Administrators

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1991-1992

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Governor of California

Leo McCarthy
Lieutenant Governor

Willie L. Brown, Jr.
Speaker of the Assembly

Bill Honig
State Superintendent of Public Instruction

Carl J. Stoney, Jr.
President of the Alumni Associations of the University of California

Paul J. Hall
Vice President of the Alumni Associations of the University of California

David P. Gardner
President of the University

Appointed Regents

William T. Bagley
Roy T. Brophy
Clair W. Burgener
Yvonne Brathwaite Burke
Glenn Campbell
Frank W. Clark, Jr.
John G. Davies
Tirso del Junco, M.D.
Alice J. Gonzales
Jeremiah F. Hallisey
S. Sue Johnson
Meredith Khachigian
Leo S. Kolligian
Howard H. Leach
Robert E. Murphy
S. Stephen Nakashima
Roy L. Shults
Dean A. Watkins
Harold M. Williams
Alex Wong
Jacques S. Yeager

Faculty Representatives

Martin Trow
W. Elliott Brownlee

Officers of the Systemwide Administration

1991-1992

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President of the University

William R. Frazer
Senior Vice President, Academic Affairs

Ronald W. Brady
Senior Vice President—Administration

William B. Baker
Vice President—Budget and University Relations

Kenneth R. Farrell
Vice President—Agriculture and Natural Resources

Cornelius L. Hopper
Vice President—Health Affairs

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President of the University, Emeritus; Professor of Business Administration, Emeritus

Charles J. Hitch
President of the University, Emeritus; Professor of Economics, Emeritus

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President of the University, Emeritus; Professor of Physics, Emeritus

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Chancellor, Emeritus; Professor of Statistics, Emeritus

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Chancellor, Emeritus; Professor of Botany, Emeritus

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Chancellor, Emeritus; Professor of Political Science, Emeritus

Dean E. McHenry
Chancellor, Emeritus; Professor of Comparative Government, Emeritus

James H. Meyer
Chancellor, Emeritus; Professor of Animal Science, Emeritus

John B. de C. M. Saunders, M.D.
Chancellor, Emeritus; University Librarian, Emeritus; Professor of Anatomy, Emeritus; Professor of History of Health Sciences, Emeritus

Robert L. Sinsheimer
Chancellor, Emeritus

Angus E. Taylor
Chancellor, Emeritus; University Provost, Emeritus; Professor of Mathematics, Emeritus

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Vice President—Budget Plans and Relations, Emeritus

Baldwin G. Lamson, M.D.
Vice President—Financial and Business Management, Emeritus; Professor of Pathology, Emeritus

Elmo R. Morgan
Vice President—Physical Planning and Construction, Emeritus

Dorothy E. Everett
Assistant President, Emeritus

Norman H. Gross
University Auditor, Emeritus

Loren Furtado
Assistant Vice President, Emeritus

Chancellors

Chang-Lin Tien
Chancellor at Berkeley

Theodore L. Hullar
Chancellor at Davis

Jack W. Peltason
Chancellor at Irvine

Charles E. Young
Chancellor at Los Angeles

Rosemary S. J. Schraer
Chancellor at Riverside

Richard C. Atkinson
Chancellor at San Diego

Julius R. Krevans
Chancellor at San Francisco

Barbara S. Uehling
Chancellor at Santa Barbara

Karl S. Pister
Chancellor at Santa Cruz

Resources Agency Sea Grant Advisory Panel

William Shafroth, Chairman
Resources Agency
1416 Ninth Street, Suite 1311
Sacramento, California

Peter Douglas
Executive Director
California Coastal Commission
San Francisco, California

Donald L. Keach
Director
USC Sea Grant Program
University of Southern California
Los Angeles, California

Senator Barry Keene
State Capitol
Sacramento, California

Assemblyman Tom Mays
2130 Capitol Building
Sacramento, California

Paul Mount
Manager, Long Beach Operations
California State Lands Commission
245 W. Broadway, Suite 425
Long Beach, California

Al Petrovich
Chief, Marine Resources Division
California Department of Fish & Game
1416 Ninth Street, 12th Floor
Sacramento, California

Gerald A. Pollock
Hazard Evaluation Section
Department of Health Services
Sacramento, California

Robert E. Ross
Executive Director
California Fisheries and Seafood Institute
Sacramento, California

Richard Sapudar
State Water Resources Control Board
P.O. Box 100
Sacramento, California

Bill Satow
Interim Director
Department of Boating and Waterways
1629 S Street
Sacramento, California

Theodore C. Smith
Department of Conservation
1416 Ninth Street, Room 1341
Sacramento, California

Fred N. Spiess
Scripps Institution of Oceanography
University of California
La Jolla, California

F. Robert Studdert
San Rafael, California

Elmer P. Wheaton
Portola Valley, California

California Sea Grant Committee

James J. Sullivan, Chairman
Director
California Sea Grant College
9500 Gilman Drive
University of California
La Jolla, California 92093-0232

John H. Crowe
Professor of Zoology
Department of Zoology
University of California
Davis, California 95616

David G. Hankin
Professor of Fisheries
Department of Fisheries
Humboldt State University
Arcata, California 95521

Robert W. Holmes
Professor, Emeritus
Department of Biological Sciences
University of California
Santa Barbara, California 93106

John R. Hunter
Chief, Coastal Fisheries Resources
National Marine Fisheries Service
8604 La Jolla Shores Drive
P.O. Box 271
La Jolla, California 92093-0203

Donald L. Keach
Director
USC Sea Grant Program
University of Southern California
Los Angeles, California 90089-1231

Michael M. Mullin
Director
Marine Life Research Group
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California 92093-0218

John S. Pearse
Professor of Biology
Institute of Marine Sciences
University of California
Santa Cruz, California 95064

Richard J. Seymour*
Research Engineer
Marine Research Division
Scripps Institution of Oceanography
University of California
La Jolla, California 92093-0222

William C. Webster
Naval Architecture and Offshore
Engineering, Room 202
University of California
Berkeley, California 94720
Berkeley, California 94720

Susan L. Williams
Associate Professor
Department of Biology
San Diego State University
San Diego, California 92182-0057

*Dr. Seymour appointed to Texas A&M for 2 years

Aquaculture Industry Advisory Committee

Mike Gafford
Namakan West Fisheries
P.O. Box 2162
Los Banos, California 93635

Richard D. Glenn
8455 Via Mallorca #41
La Jolla, California 92037

George Lockwood
Ocean Farms Hawaii
P.O. Box A
Kailua-Kona, Hawaii 96745

Thomas B. McCormick, III
McCormick & Associates
323 E. Matilija Street #112-131
Ojai, California 92023

John McMullen
Ab Lab
Naval Civil Engineering Lab
Port Hueneme, California 93043

Frank Oakes
The Abalone Farm
P.O. Box 136
Cayucos, California 93430

Hugh W. Staton
Abalone Unlimited, Inc.
2455 Jacaranda Lane
Los Osos, California 93402

Peter Struffenegger
Sea Farms California
P.O. Box 99
Herald, California 95638

F. Robert Studdert
36 Professional Center Parkway
San Rafael, California 94903

Philip L. Wilson, III
Aquaculture Enterprises
P.O. Box 3314
Kailua-Kona, Hawaii 96745

Seafood Industry Advisory Committee

Tod Ghio, Chairman
Ghio Seafood Products
5232 Lovelock Street
San Diego, California 92110

Joe A. Caito
Caito Fisheries, Inc.
P.O. Box 1370
Fort Bragg, California 95437

Maurice Camillo
J. J. Camillo Seafood Brokerage
545 Harbor Lane
San Diego, California 92101

Tom Elliott
TEMA, Inc.
P.O. Box 3894
San Francisco, California 94119

Marty Greenwald (alternate)
Ocean Garden Products
520 N. Brookhurst Street, Suite 200
Anaheim, California 92801

Thomas B. McCormick, III
McCormick & Associates
323 E. Matilija Street #112-131
Ojai, California 93023

Bill Merry
W. R. Merry Seafood Company
636 Stanford Avenue
Los Angeles, California 90021

Wayne Miller
4454 Olive Hill Road
Fallbrook, California 92028

William Perkins
Western Fishboat Owners Association
P.O. Box 926
Dana Point, California 92629
Art Haworth (alternate)
P.O. Box 8978
Incline Village, Nevada 89450

J. David Ptak
Chesapeake Fish Company
535 Harbor Lane
San Diego, California 92101

Ray Swanson
Swanson Brothers Seafood
30 Pamaron Way, Suite 203
Novato, California 94949

Nickolas A. Vitalich
Chesapeake Fish Company
535 Harbor Lane
San Diego, California 92101