CALIFORNIA SEA GRANT

Biennial Report of Completed Projects 1984–86

A Publication of the California Sea Grant College Program

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The California Sea Grant College Program is a statewide, multiuniversity program of marine research, education, and advisory services, administered by the University of California Institute of Marine Resources. 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 nation. 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 in California and throughout the nation. Sea Grant also supports a broad range of educational programs for university students, public school teachers and students, and the general public 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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California Sea Grant College Program
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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 1984-85 and 1985-86. It is meant to be a technical record of our accomplishments for use by individuals in academia, government, and industry. Unlike earlier biennial technical reports in the series, it 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 Program is administered by the University of California Institute of Marine Resources, located at Scripps Institution of Oceanography on the University of California, San Diego campus. Policy guidance comes from the Advisory Council of the Institute of Marine Resources, appointed by the president of the University of California. The California Sea Grant Committee, composed of representatives from the University of California and State University Systems and private universities, provides administrative guidance on matters pertaining to the conduct of the Sea Grant program and the pursuit of its objectives. The committee also reviews the program subject areas and appoints independent review panels to assist it in this task.

A seafood industry advisory committee, an aquaculture industry advisory committee, and several other committees help in creating program policy. The Resources Agency Sea Grant Advisory Panel provides valuable program planning and development efforts to help Sea Grant identify and meet state needs.

James J. Sullivan
Director

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Coastal Resources

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Phytoplankton in Eutrophic Coastal Waters

Raymond C. Smith

University of California, Santa Barbara
R/CZ-64
Project Initiated: October 1, 1984
Project Completed: September 30, 1985

In the oceans, the fundamental ecological process is the conversion of solar radition into biochemical energy by free-floating plants called phytoplankton. The rate of this process is called primary production, and this plant life is an essential component in the ocean's production of all organisms, including fish and marine mammals, further up the food chain.

A major objective of this Sea Grant research was the collection, organization, and utilization of combined ship, satellite, and other ancillary data for the purpose of better understanding the dynamics of phytoplankton in the Southern California Bight region. In turn, these phytoplankton distributions were related to the abundance and distribution of other marine organisms.

The training of graduate students in the relatively new skills of managing, analyzing, and using ship and satellite data for research was also a major objective of this Sea Grant project.

As a result of this project, Randee Recht has completed her master's thesis, "Geographical Registration of Multiplatform Oceanographic Data." This work provides a basis for the direct intercomparison and utilization of diverse and disparate data sets and the systematic data-base management of these various data. A major impediment to the widespread usage of satellite data has been the difficulty of effectively utilizing these (by normal standards) very large data sets in conjunction with more familiar data. This work provides a methodology for merging and effectively utilizing these data.

Xueyun Zhang has completed her Ph.D. dissertation, which deals with the regional mapping of chlorophyll concentrations in the Southern California Bight and the physical processes influencing the distributions of phytoplankton biomass. Several years of satellite and contemporaneous ship data have been screened and processed, ancillary data sets have been organized into consistent formats for comparison with the satellite imagery, and these combined data sets analyzed.

Elisabeth Dunlap completed a master's thesis, "Abundance and Distribution of Cetaceans in the California Current System as Observed from Ship and Satellite Data." This work provides evidence that sea-surface temperature and chlorophyll concentration, both of which can be quantitatively estimated using satellite sensors, can be used as "habitat descriptors" for various species of cetacea. In turn, these satellite-determined habitat descriptors can then be utilized to optimize cetacea studies and estimates of their abundance and distribution.

A major accomplishment of this project was the integration and data-base management of disparate data from various sources and the effective application of these data to a practical problem.

As an example, shipboard sightings of marine mammals, ship-continuous data logging of sea surface temperature, satellite sea-surface temperature data from the NOAA-AVHRR sensors, and chlorophyll concentrations derived from the Nimbus-7 CZCS were used to formulate a new methodology for optimizing marine mammal studies and estimating their abundance and distribution. Analogous work with respect to phytoplankton dynamics is currently under study.

Cooperating Organizations
National Aeronautics and Space Administration
National Science Foundation
Office of Naval Research
University of California Marine Bio-Optics Group

Development of a Remote Sensing Aided Procedure for Water Quality Monitoring

University of California, Berkeley
University of California, Davis
R/CZ-68
Project Initiated: October 1, 1984
Project Completed: March 30, 1986

Robert N. Colwell, Allen W. Knight, and Siamak Khorram

The major goal of this research effort was to develop models based on two Landsat MSS overpasses and compare the applicability and reliability of developing models for one date and applying them to another date over the same geographic areas. The second goal was to determine a series of spectral characteristics for a high biological activity area, called the entrapment zone, and consequently determine the location and areal extent of this zone. A third objective was set for this project by virtue of having airborne Daedalus MSS 1260 data available. This objective should be considered as a bonus to this project. This objective was to develop regression models for predicting chlorophyll *a* concentrations based on one date for application to another date over the same geographic area.

Landsat MSS Studies

Predictive multidate water quality models based on Landsat MSS reflectance values were developed for 1980 and 1983 turbidity (TURB), total suspended solids (TSS), and salinity (SAL) concentrations in the San Francisco Bay and Delta. These models were tested for statistical significance, goodness of fit, and structural consistency to determine if generalized models could characterize both dates given differences in delta flow conditions, tide phases, satellite sensors, and sun elevations.

Water quality data covering the bay and delta were collected within 1 hour before and after overpasses of Landsat 2 (1980) and Landsat 4 (1983) under near-slack and flood tides, respectively. The 1980 delta flow conditions were normal for the season, while 1983 flow was extremely high and similar to spring runoff. Literature sources and preliminary analysis suggested that the bay and delta were hydrologically distinct and that Landsat MSS could detect differences in hydrodynamic conditions.

Tests of models representing the entire area indicated that different models were required for each date. Comparison of regional models suggested that generalized TURB and TSS functions could explain conditions for both dates only within the delta. Delta models were applied to their respective MSS data, and color-coded maps identifying discrete concentration levels were generated. The imagery was checked against water quality sample data indicating entrapment zone characteristics, and locations of both entrapment zones were compared.

Airborne Scanner Studies

Two airborne scanner studies examining the relationship between near-surface measured chlorophyll *a* values and reflectance as measured by a Daedalus 1260 Multispectral Scanner have been completed. The first involved the simultaneous acquisition of surface chlorophyll *a* concentrations from boats and Daedalus 1260 Multispectral Scanner data from a U-2 aircraft. It was conducted in the northern reaches of San Francisco Bay on August 28, 1980. These data were used to develop regression models for predicting surface chlorophyll *a* concentrations over the study area for ebb tide (8:40 a.m. PDT) and flood tide (3:10 p.m. PDT) conditions on a 40 x 40 m resolution basis.

The objectives of this study were to determine the following:

1. Whether a scanner similar to the Thematic Mapper aboard Landsat could provide data useful to the detection of chlorophyll *a*;
2. What wavelength regions were the most important indicators of varying chlorophyll *a* concentrations;
3. What mathematical models describe the most consistent statistical relationship between scanner and surface measured data; and,
4. If the coefficients of these linear regression models are conservative from ebb to flood tide.

The two channel combinations employed as independent variables in regression modeling were Daedalus channel 3 (450–500 nm) minus Daedalus channel 10 and Daedalus channel 7 (650–690 nm) divided by Daedalus channel 8 (700–790 nm). Channels 3 and 7 represent chlorophyll *a* absorption wavelengths, and channel 8 covers wavelengths dominated by chlorophyll *a* reflectance. Subtracting channel 10 (920–1100 nm) from channel 3 is an attempt to reduce the variability in scanner data due to haze backscatter by removing a value that mainly represents atmospheric reflectance, since most of the energy reaching the water surface in those longer wavelengths were absorbed.

There were six major steps employed in the production of chlorophyll *a* predictions for ebb and flood tide:

1. Simultaneous acquisition of Daedalus 1260 Multispectral Scanner and surface truth measurements for 39 sample sites.
2. Laboratory analysis of water samples filtered through glass fibers, extracted in 95% acetone and measured fluorometrically. Chlorophyll *a* values used in modeling were corrected for phaeopigments;
3. Acquisition of Daedalus data

on magnetic tapes. These data were registered to a Universal Transverse Mercator (UTM) grid system and the count values for pixels designated as those encompassing the surface sample sites extracted.

4. Replacement of bad data with interpolation (as in the case of on-scan line offset) or removal if the bad data included more than one missing scan line.

5. Development of mathematical models. Using linear regression techniques, the surface-measured chlorophyll *a* values were input as the dependent variable, while the scanner count values were entered into the regression as the independent predictor variable. Of the 39 sample sites 30 were used for model development in both ebb and flood tide data sets. The remaining 9 sites were run through the model after development as verification. The only constant between the ebb and flood tide models is in the use of the same Daedalus channel combinations (channel 3 minus 10 and channel 7 divided by 8) to predict surface chlorophyll *a*. Criteria examined to compare various models included R^2 values, *F*-test results of the significance of the overall model, *t*-test results of the significance of the coefficient estimates, standard errors of the overall model and each of its coefficients, and residual analysis.

6. Production of final color-coded maps of surface chlorophyll *a* predictions. Ebb and flood tide spectral data were run through selected linear equations producing pixel-by-pixel predictions of chlorophyll *a*. These continuous predictions were grouped into discrete concentration classes and were assigned colors yielding a map of surface chlorophyll *a* for the entire study area.

The results include the spectral and surface sample data sets, sets of linear equations, and color-coded maps of chlorophyll *a* predictions for ebb and flood tide conditions. The resolution afforded by the Daedalus scanner allowed for greater discrimination of local patchiness of chlorophyll *a*. Spectral vs. surface measurements regression performed on a subset of 15 sample sites yielded a multiple correlation coefficient (R^2) of 0.82, while regression using ship-board fluorometric trace vs. surface measurements with the same data subset produced an R^2 of 0.90.

Conclusions drawn from the results of this investigation indicate the following:

1. A multispectral scanner similar to the Thematic Mapper can be used to map surface chlorophyll *a*.

2. Blue, red, and near-infrared wavelength regions contain information necessary to produce chlorophyll *a* predictions (Daedalus 1260 MSS channels 3, 7, and 8).

3. The morning (ebb tide) model measured surface chlorophyll *a* = 323.7 + 2.4 (channel 3 minus 10) – 299.2 (channel 7 divided by 8), and the afternoon (flood tide) model measured surface chlorophyll *a* = 570.8 + 3.1 (channel 3 minus 10) – 541.2 (channel 7 divided by 8). Both models explain a statistically significant proportion of the variation in surface measured chlorophyll *a* data.

4. Some sort of standardization for contributing environmental factors will be necessary in the future if the desire to apply these models ranges beyond the geographic region for which they were developed.

The second study was conducted on September 13, 1983, during ebb-to-ebb slack tidal conditions. Ten visible and near infrared wavelength channels were digitally recorded by an airborne Daedalus multispectral scanner over the northern portion of the San Francisco Bay system. The scanner was flown over the study area aboard a NASA U-2 aircraft at an altitude of 70,000 feet between 11:16 and 11:24 a.m. PDT with a ground resolution of approximately 28 m. Surface water samples for 34 sites throughout the 10 x 25 square mile study area were acquired between 10:30 a.m. and 12:43 p.m. PDT. The 34 samples were analyzed for salinity, turbidity, total suspended solids, and chlorophyll *a* concentration.

The objective of this investigation was to examine the applicability and consistency of a previously developed model reflecting the relationship between digital spectral data and the concentration of chlorophyll *a* in this estuarine environment. This research will explore the possibility of developing models that can apply under different environmental conditions in the same geographic area. Locating 34 sample sites as pixel coordinates on scanner imagery was accomplished by the use of 55 control point locations throughout the study area transferred from NOAA nautical charts using a second-order polynomial function. Pixels identified as sample site locations in this fashion became the center of a 5 x 5 pixel matrix. The average digital spectral values from these matrices were used to represent the sample sites. Each of the 34 sample site matrices were examined for widely fluctuating spectral values. All digital spectral values for each matrix were within ±0.5% of their matrix mean, and there were no bad data anywhere within the study area.

Previous research had indicated that the 475-nm, 670-nm, 745-nm, and 1010-nm wavelength channels of the Daedalus 1260 MSS contained spectral information related to near-surface chlorophyll *a* concentration. Previous work had also highlighted the need for surface measurements to be made as near as possible to overpass duration, especially in a dynamic estuarine study area. Spectral values for each of the 34 sample locations were transformed into two wavelength ratios: 485 nm/980 nm and 660 nm/720 nm. These ratio values were employed as independent variables in linear regression with surface measured chlorophyll *a* values.

Results indicated that the use of

these ratios as predictor variables is dependent on the magnitude of the chlorophyll *a* concentration as well as on the length of time between scanner overpass and surface data collection. The 485 nm/980 nm ratio appears to function most consistently in this application when near-surface chlorophyll *a* concentrations are <15–20 µg/liter. On the other hand, the 670 nm/745 nm ratio used in previous research was the best predictor variable when near-surface chlorophyll *a* concentrations for sample sites ranged from about 20 to 80 µg/liter. Development of the final model used to produce a color-coded spatial display of chlorophyll *a* concentration for the entire study area was based on surface data recorded within 15 minutes of scanner overflight time.

Predicted spatial patterns of chlorophyll *a* were very different from patterns produced during previous research. This difference is driven primarily by the magnitude of freshwater inflow into the northern portion of the San Francisco Bay system, since both previous and current research were conducted at the same time of year. Freshwater inflow in September 1983 was a record 20,000 cubic feet/second. This high volume was accompanied by low chlorophyll *a* concentrations noted during surface observations in areas of high phytoplankton biomass during normal inflow years (5,000–10,000 cubic feet/second) in this and other surface-sampling studies. With sufficient calibration, remotely sensed data may be able to provide synoptic views of phytoplankton dynamics difficult to obtain from surface point or trace sampling alone.

The results of these two studies support the contention of experts on phytoplankton dynamics that the location of the chlorophyll *a* maximum (indicative of high biological productivity) is a function of the freshwater inflow characteristics. Application of models to future spectral data acquired over the northern portion of the San Francisco Bay estuary may help to further substantiate or refute present theories of estuarine dynamics based on surface point sampling and provide synoptics of current estuarine condition.

Cooperating Organizations

California Department of Fish and Game, Stockton
California Department of Water Resources, Sacramento
National Marine Fisheries Services, Tiburon
North Carolina State University, Computer Graphics Center, Raleigh
U.S. Bureau of Reclamation, Sacramento
U.S. Geological Survey, Water Resources Division, Menlo Park

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Coastal Engineering Implications of Trends and Fluctuations in California Coastal Sea Level

University of California, San Diego
R/CZ-69
Project Initiated: October 1, 1983
Project Completed: November 30, 1985

Reinhard E. Flick

The overall project objective was to identify and quantify the important processes that affect sea level on the California coast in a manner that could be applied to the assessment of coastal flooding risk. The study was stimulated by the widespread damage exceeding $100,000,000 that occurred in California during the winter of 1982–83. The severe flooding was a result of a coincidence of unusual oceanographic and meteorological events including peak high tides, a strong, 2-year El Niño condition, and persistent meteorologically forced storm surge. Emphasis in the research was on two related subobjectives:

1. to examine the statistical relationships of local wind and atmospheric pressure fluctuations to local sea level changes on time scales shorter than 1 month, and
2. to study extreme events such as the winter of 1982–83 in detail to attempt to quantify the relative size of local forcing and large-scale influences, namely, the symptoms of the Pacific Ocean-wide El Niño phenomenon.

The height of sea level along coastal regions is the result of the astronomical tide-producing forces, along with additional effects of the ocean, such as currents, density fluctuations, and meteorological forcing by wind and atmospheric pressure. Tides dominate the shortest time scale changes, with meteorological and steric effects typically becoming important on scales of days to months. Tides along the coast of California are of the "mixed" type, with diurnal constituents of the same order of magnitude as the semidiurnal components. This is crucial for explaining the nature of the extreme range of tides (Zetler and Flick, 1985a,b). The effect of tropic tides dominates the extremes so that maximum tides occur in summer and winter. This maximizes the likelihood of coincidence of high tide levels with storm-induced sea level extremes during the winter storm period along California (Figure 1).

In general, tide gauge records in California show a secular rise in relative sea level over the last 80 years, presumably due to large-scale climatic variations. If this trend continues, any future anomalous high sea and storm episodes will be further amplified. Flick and Cayan (1984) showed the tendency for such extremes to occur in runs during large-scale anomalous winters (see Figure 2).

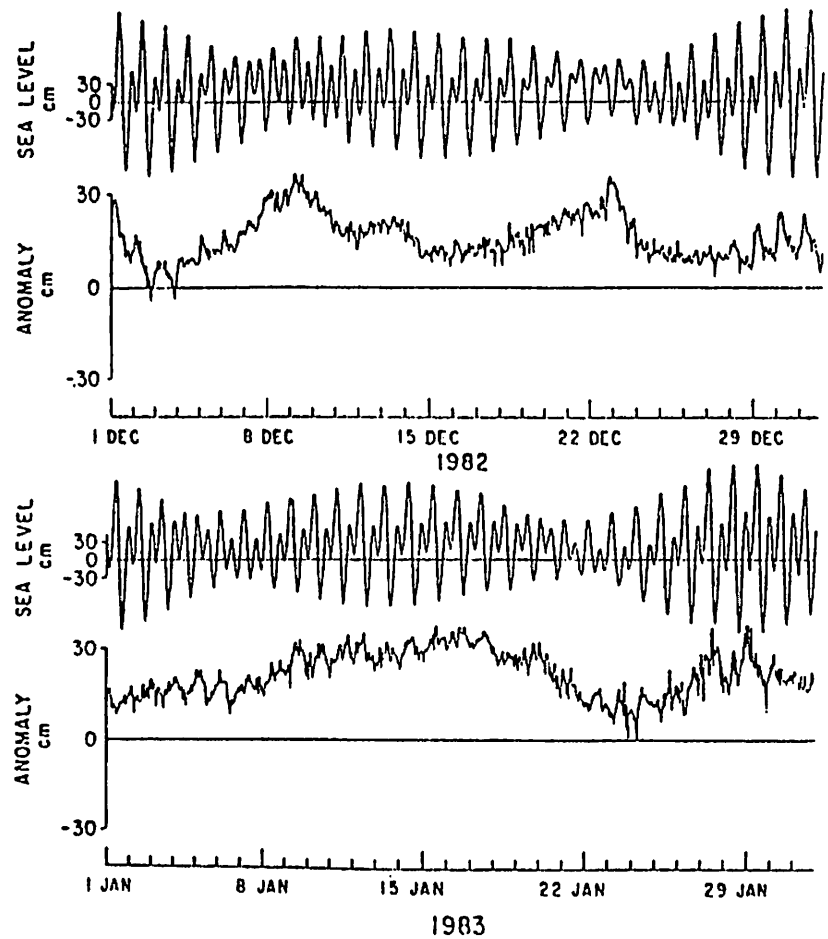

Figure 1. Measured sea level and unfiltered anomaly for December 1982 and January 1983 at San Diego. Anomaly computed by subtracting harmonic tide prediction from measurements. Note coincidence of large anomalies related to storm passage with neap tides in December 1982, but with larger, spring/tropic tide in late January 1983.

To examine the effects of mechanisms other than tides and long period secular rise, measured sea level extremes were adjusted by subtracting the tide prediction and the secular trend (about 18 cm/century) at San Diego. Statistical analyses of the adjusted anomaly data show that the return period for 0.3-foot anomalies is about 2 years. Anomalies of 0.5 foot occur on average about every 4 years. The longer interval, larger anomalies have not recurred often enough to derive stable statistics, and not too much faith should be put in their return period. On the other hand, anomalies on the order of 1.0 foot are certainly possible because they have been observed at San Diego and at other stations on the California coast.

For coastal engineering purposes it is highly desirable to have a direct, predictive relationship between coastal sea level response and the atmospheric forcing variables. This could be viewed as a statement of the storm surge problem. Unfortunately, the relationship between coastal sea level fluctuations and the atmospheric forcing by pressure and wind in the band between seasonal and inertial/tidal frequencies is very complex. Correlation, spectral, and multiple linear regression analysis methods have been applied to edited and filtered hourly time series of sea level and meteorological variables available from Scripps pier and San Diego Bay.

Tidal fluctuations were substantially removed from the sea level records by subtracting a standard harmonic prediction. Residual tidal noise and all other higher frequency signals in the sea level and atmospheric data were removed by digitally filtering to pass only the 2 to 30-day band. Filtered signals were decimated to one sample per day, and the 608-day time period from 1 January 1982 to 31 August 1983 was separated for further study (Figure 3). Inspection of the time series shown in Figure 3 shows large fluctuations in the sea level anomaly during the November 1982 to April 1983 winter. Peak values range up to 20 cm. Storms occurred an average of about three times per month, with peak wind speeds on the order of 15 m/sec and atmospheric pressure anomalies over –10 mbars and with usual durations of 3–5 days (Cayan and Flick, 1985).

Correlation and spectral analysis suggests that the primary response of sea level anomaly is to the barometric pressure in the 2 to 30-day band. Simple cross-correlation functions have been computed between anomaly and pressure. The correlation peaks at about –0.8 at zero lag, which is qualitatively consistent with inverse barometer response. The regression factor is found to be about –1.2 cm/mbar, suggesting an additional minor sea level response to forcing itself coherent with the pressure, or some shelf-wave activity leading to increased response due to weak resonances. Positive anomalies are associated with south wind, which is qualitatively consistent with cross-shelf geostrophic balance.

Multiple linear regression analysis of the hourly La Jolla winter data shows that over 60% of the variance in sea level anomaly can be related to pressure and wind with significant skill. A "predicted" anomaly can be written in the form

$$\hat{\alpha}(t) = \alpha p(t) + \beta u(t) + \delta v(t - 18)$$

where α, β, and δ are regression coefficients and t is time in hours. The resulting correlations $\hat{\alpha}p = -0.9$, $\hat{\alpha}u = 0.2$, and $\hat{\alpha}v = 0.5$ suggest that pressure is the primary agent, followed by longshore wind and finally on-offshore wind. It is common for longshore winds to be more effective than on-offshore winds at producing sea level fluctuations at subinertial periods (Allen, 1980; Mysak, 1980a,b).

Cooperating Organizations

California Department of Boating and Waterways

The Quest for Truth Foundation, Seattle, Washington

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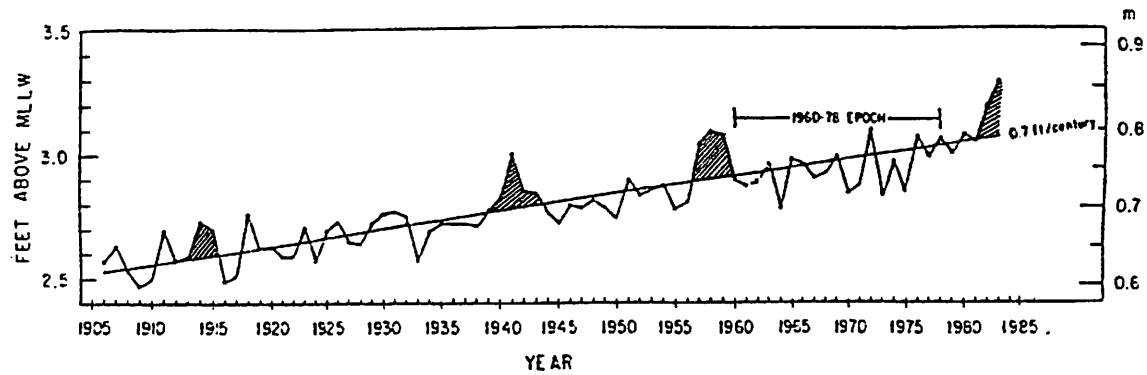

Figure 2. Yearly mean sea level about 1960–78 MLLW datum at San Diego. Straight line shows secular increase of 0.7 foot/century, comparable to global sea level rise. Shaded areas are coincident or near in time to major El Niño episodes.

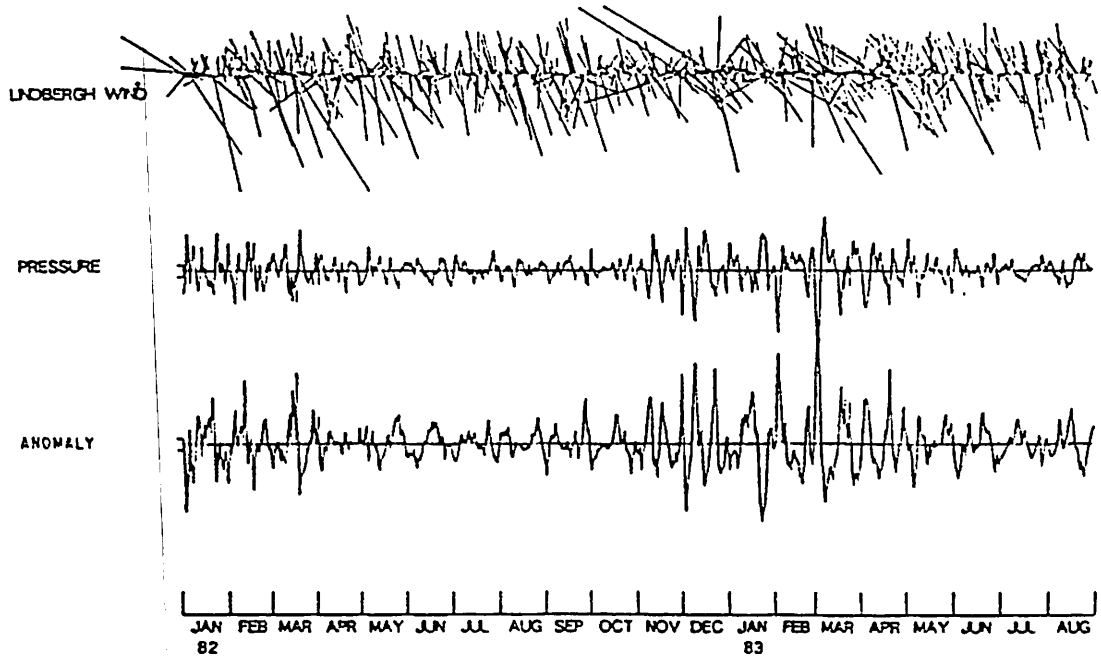

Figure 3. Time series of daily wind vector (upper), atmospheric pressure (middle), and sea level anomaly (bottom) at San Diego (1982–83). Wind direction follows compass heading, i.e., sticks from upper left indicate wind from northwest. Maximum length sticks indicate speed at about 15 m/sec. Ticks on pressure and anomaly axis show ±1 mbar and ±1 cm, respectively.

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Wave Groups in Surface Gravity Waves

University of California, Berkeley
R/CZ–70
Project Initiated: October 1, 1984
Project Completed: September 30, 1986

Rodney J. Sobey

In line with the project objectives, the research focused on the nature and extent of wave grouping. Development of models was not pursued.

The basis of the research was an extensive data base of wave records in moderately deep water from a wide range of wave climate conditions. The data included Tasman Sea storm waves off Botany Bay and Newcastle on the eastern coast of Australia, winter Southern Ocean swell in the Great Australian Bight, and hurricane waves on the North West Shelf. These were to be supplemented by winter storm waves in California near the Farallon Islands, but this did not eventuate because of incompatible magnetic tape formats. Budgeted items for microcomputer support were not funded by the California Sea Grant College Program, and data base establishment was very time consuming.

The development of record analysis algorithms was based on the wave record itself and on its complex envelope function, as well as on linear wave theory and linear time series analysis techniques. Wave record analysis in the frequency domain concentrated on the complex Fourier transform of the wave record. The Gaussian random wave model assumes the phase spectrum to be completely random, but it does possibly contain some useful information. Two problems were identified in interpretation of the phase spectrum—phase unwrapping and phase trend removal—which contribute to the apparently random character of computed phase spectra (Read and Sobey, 1987).

Phase unwrapping refers to the modulo 2π operation on phase angles. The phase returned by the Fast Fourier Transform algorithm is the principal phase, in the range $-\pi$ to π. Any principal phase may in fact be the principal phase plus $2\ell\pi$, where ℓ is any signed integer, without changing either the complex Fourier transform or the variance spectrum. A unique value of the integer is nominally available from the slope of the phase spectrum (Read and Sobey, 1987). In principle, integration of the phase slope should yield the unwrapped phase spectrum, but there are a number of problems in practice. The phase-slope estimates are raw estimates and significantly complicate the numerical interaction. Some theoretical assistance is available from the raw principal-phase estimate, and advantage has been taken of both estimates. A further and more significant problem is the specification of the phase and phase gradient at zero or near-zero magnitude points of the Fourier transform, including the initial conditions. These singular points can be accommodated by classical limit theory (L'Hospital's rule) as demonstrated by Read and Sobey (1987). Figure 1 is a typical analysis result, showing the raw and smoothed variance spectrum together with the raw and smoothed unwrapped phase spectrum. A linear trend component, corresponding to an origin shift in time for the wave record, is routinely removed from the unwrapped phase spectrum.

Wave-record analysis in the time domain focused attention on the correlogram tail. If the phase spectrum is indeed (narrow band) random, as required by the Gaussian random-wave model, the envelope of the correlogram should go asymptotically to zero in about five wave periods. This is not typically observed, and the dominant feature is the ordered tail. This may be a wave-group signature, but it may also be a spurious computational effect: it is not easily dismissed, however. Kendall and Stuart (1966) give the 95 percent confidence limits of the correlogram tail for a random process as -0.00 ± 0.04 for a 2048 point-wave record. The amplitude of the envelope in the correlogram tail is typically 0.15, well outside the 95 percent confidence limit for a random process.

The complex envelope function A(t) is a potentially attractive analysis vehicle for the identification of wave grouping. It is related to the wave record as

$$\eta(t) = Real\ [A(t)\ \exp\ (i\omega_o t)]$$

where ω_o is a carrier frequency. No information is lost from the original record, and attention is focused on the envelope modulations. The estimation of A(t) initially follows Sobey and Colman (1983). It involves the estimation of the discrete Hilbert transform from the wave record using the FFT and inverse FFT algorithms and the definition of the pre-envelope function. This requires identification of the carrier frequency $\omega_o = 2\pi f_o$, which is initially approximated by the peak frequency $\omega_p = 2\pi f_p$. Following Bolt and Brillinger (1979), f_p can be adjusted to f_o in terms of any linear trend in the phase record. Estimation of this correction and interpretation of the phase record requires unwrapping of the principal phase. Again any principal phase may in fact be the principal phase plus $2\ell\pi$, where ℓ is any signed integer, without changing the complex envelope function. The algorithm adopted previously for unwrapping the phase spectrum in the frequency domain was adapted to the physical domain. The integer ℓ is determined from the raw principal phase and an additional raw estimate from time integration of

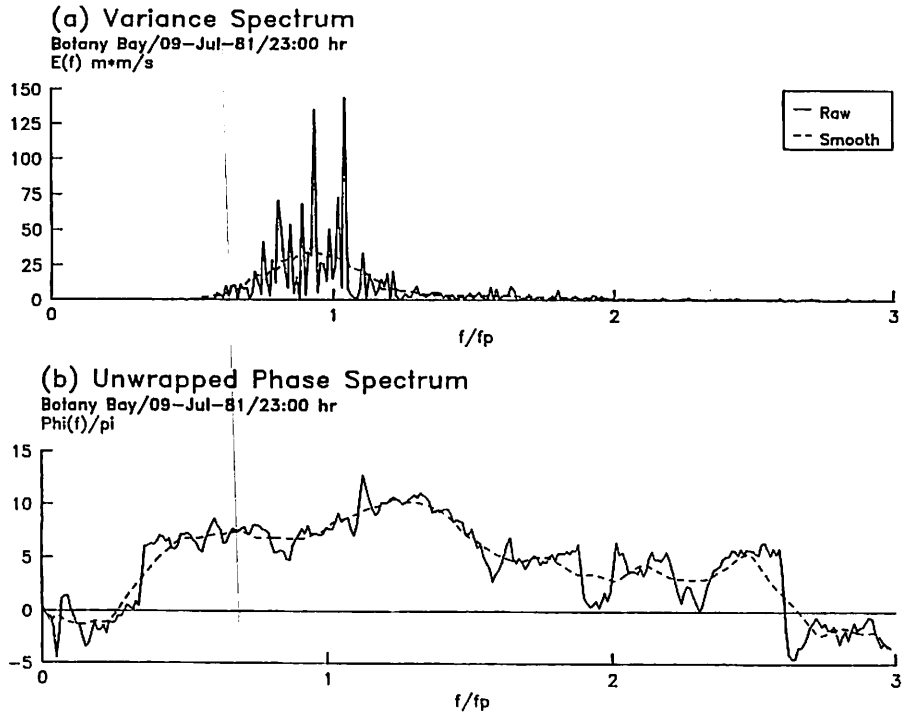

Figure 1. Typical variance and phase spectrum of wave record.

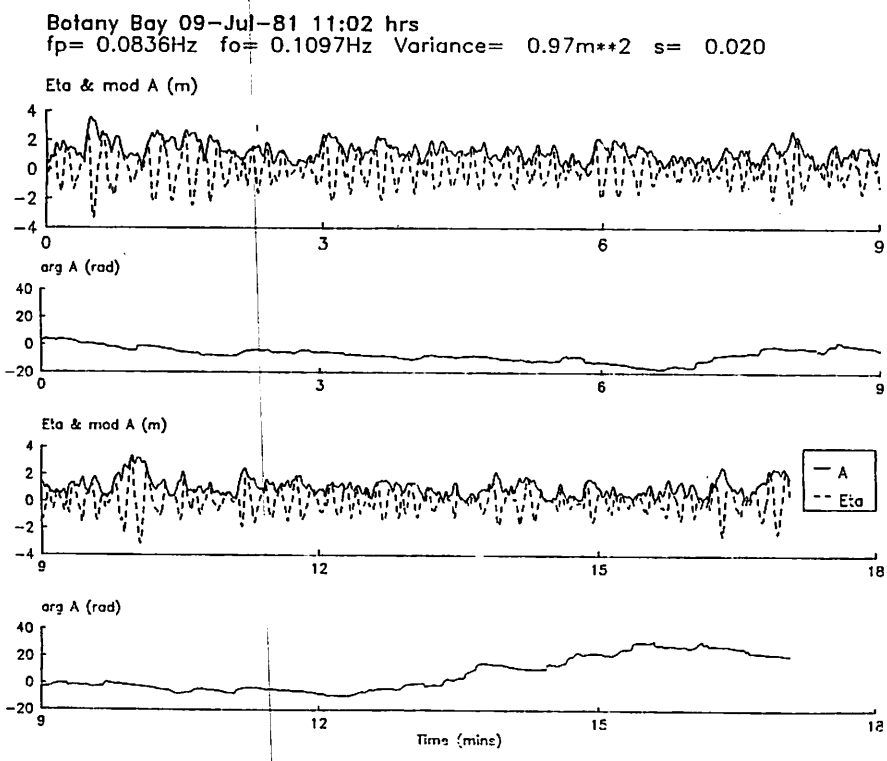

Figure 2. Typical complex envelope function.

the slope of the phase record (Sobey and Liang, 1987).

Figure 2 is a typical complex envelope result. Figure 2 shows the complete 1024 s wave record together with the corresponding mod A(t) and unwrapped arg A(t) record. Note in particular the relative ease of identification of wave groups in the mod A(t) record, the Rice envelope function. For typical narrow-banded sea state, mod A(t) is a good representation of the wave envelope and the common R_H, j_1 and j_2 statistics are readily extracted (from the correlogram). Wave grouping perhaps implies some linkage between the mod A and arg A records for which the appropriate analysis techniques are cross-spectra and cross-correlations. The removal of the dominant frequency from the record shifted the spectral peak downwards by an order of magnitude, to the extent that frequency resolution from a typical 1024 s record was quite marginal. Resolution could be improved by using a longer record, provided that it remained reasonably stationary. Records of several hours' duration are inevitably nonstationary as a result of sea state generation and evolution, but records of order of one hour, although not commonly available, proved to be a reasonable compromise. The frequency resolution is improved, though still marginal, and the record will remain reasonably stationary, except in extreme circumstances. Further analysis was based on a number of nominal one-hour (8192 point) records.

Figure 3 (Sobey and Liang, 1987) is a typical time domain analysis result from the complex envelope showing the correlograms of the mod A and arg A traces, together with the cross-correlogram for positive and negative lags. Also included on these correlograms are the 95 percent confidence bands on a random process (Jenkins and Watts, 1968). The long period cycles in all traces are indicative of wave grouping. These cycles extend sufficiently beyond the 95 percent confidence bands to require

recognition but are not sufficiently extreme to challenge the commonly-assumed narrow-band random structure of typical sea states.

Figure 4 (Sobey and Liang, 1987) is a typical frequency domain analysis result from the complex envelope, showing the raw and smoothed variance spectra of the mod A and arg A traces, together with the squared coherence and cross-phase spectra. Also included on these spectra are the 95 percent confidence bands (Jenkins and Watts, 1968) for the variance spectra and the 95 percent confidence limit on zero coherence for the squared coherence spectrum. The variance spectra shows the typical concentration of variance at low frequencies, as does the coherence spectrum. As for the time domain analysis, however, coherence levels again extend sufficiently beyond the 95 percent confidence limit to require *recognition* but are not sufficiently extreme to challenge the commonly-assumed narrow-band random structure of typical sea states.

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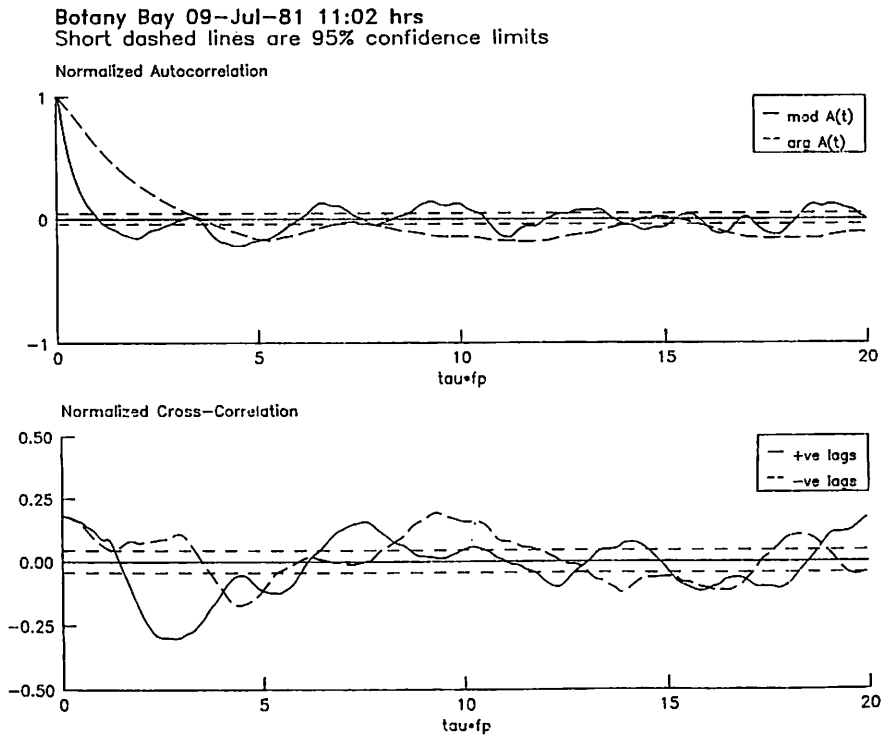

Figure 3. Typical correlograms of complex envelope function.

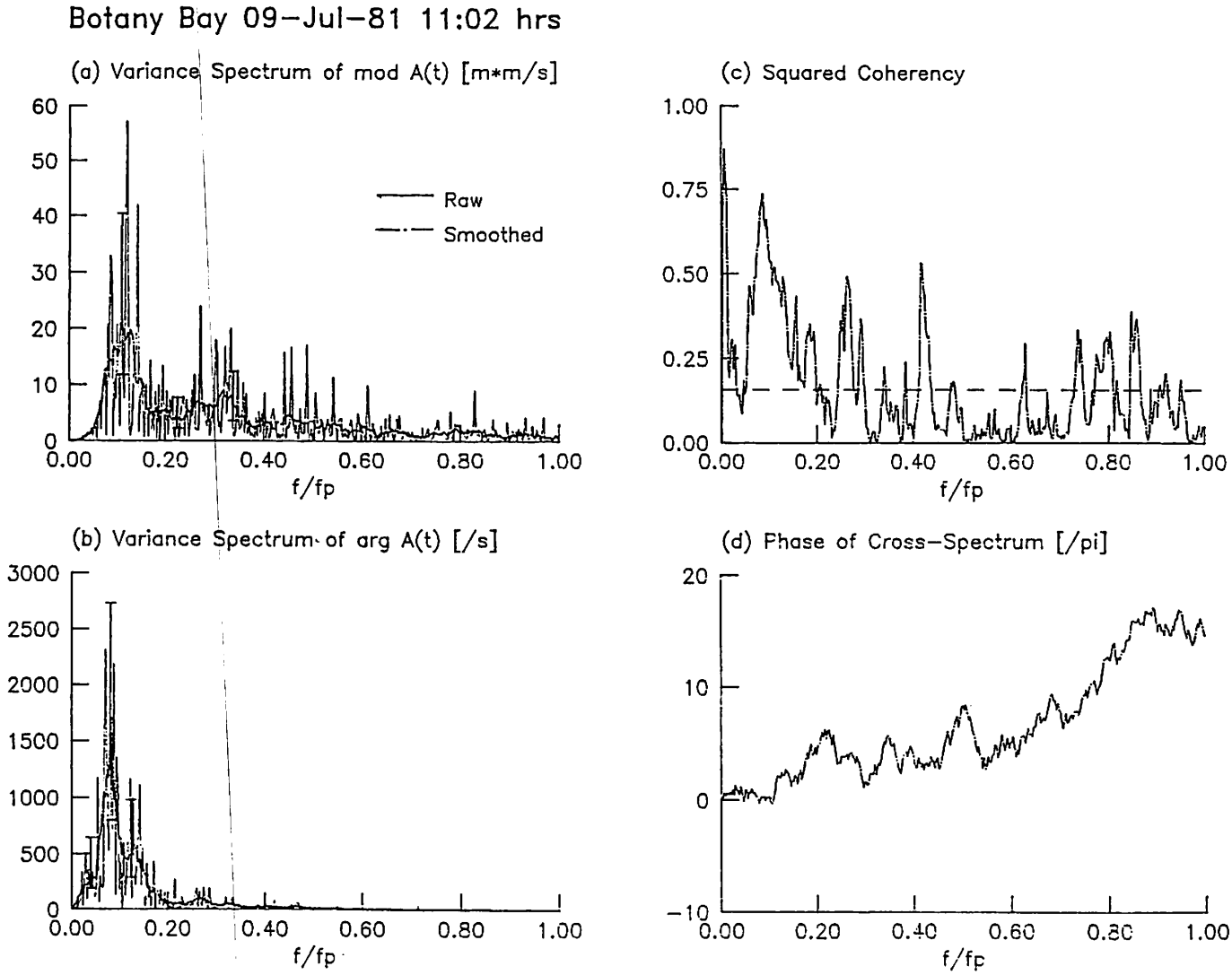

Figure 4. Typical spectra of complex envelope function.

Liquefaction Susceptibility of Fiber-Reinforced Coastal and Offshore Fills

San Diego State University
R/CZ-72
Project Initiated: October 1, 1984
Project Completed: September 30, 1985

Iraj Noorany and Michael Uzdavines

On the coast of California and in other coastal regions there are many landfills made of dredged sand placed under water without compaction. These types of fills are typically loose, and in seismically active areas they are susceptible to the danger of liquefaction. Although some post-construction soil densification techniques, such as vibroflotation, have been used to avert soil susceptibility to liquefaction, no effective soil improvement technique during the fill placement under water has been found. This research project concerns the use of synthetic fibers in sand as soil reinforcement for the purpose of improving sand's resistance to liquefaction.

Scope of the Study

A feasibility study of the liquefaction behavior of sand reinforced with fabrics was carried out during 1982–83 under the sponsorship of the California Sea Grant College Program. The results of that study (Noorany, 1984) showed that inclusion of layers of certain types of fabric had some beneficial influence on the behavior of saturated sand subject to repeated loads simulating earthquake conditions. The present study (1984–85) was aimed at investigating the influence of randomly distributed fabric strips and fibers in sand on the sand's liquefaction resistance. It was also intended to explore the feasibility of placing a mixture of sand and fibers under water.

Methods of Investigation

A literature survey was made on the subjects of sand liquefaction and the general behavior of reinforced soils. It was concluded that no previous work existed regarding the dynamic behavior of reinforced sands. In order to investigate the dynamic behavior of a composite material consisting of sand and reinforcement inclusions, 206 cyclic triaxial tests were performed both on unreinforced Monterey No. 0 sand specimens and on specimens reinforced with four commercially available products used as inclusions. The four reinforcement materials were used in nine different configurations within the reinforced specimens. These configurations ranged from four discrete layers of fabric disks, to fifteen layers of fabric strips, to randomly distributed polymeric fibers. Detailed test methods and procedures are presented in Uzdavines, 1987.

All test samples were prepared by a moist tamping technique at a relative density of 50 percent. The density of the sand phase was kept constant in all samples, both those with and those without inclusions. Results were analyzed by comparing the number of cycles of stress application required to cause initial liquefaction. Also, the patterns of deformation in unreinforced and reinforced samples were studied.

Studies related to evaluation of the feasibility of placement of composite sand-fiber material under water included a general review of current techniques of dredged fill construction as well as small-scale model tests in a water tank in the laboratory.

Summary of Results and Conclusions

Complete test results and data analysis are given in Uzdavines (1987). Figure 1 is a summary of the dynamic triaxial tests showing the relationship between the cyclic stress ratio and the number of cycles required for initial liquefaction. This figure depicts a

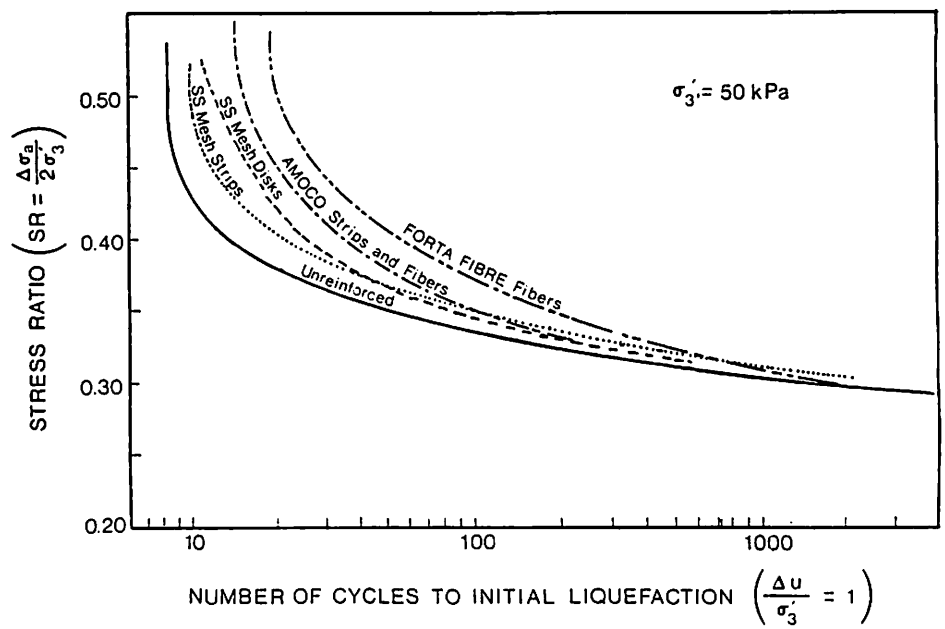

Figure 1. Comparison of stress ratio vs. number of cycles to initial liquefaction obtained from cyclic tests on unreinforced specimens and specimens reinforced with various materials.

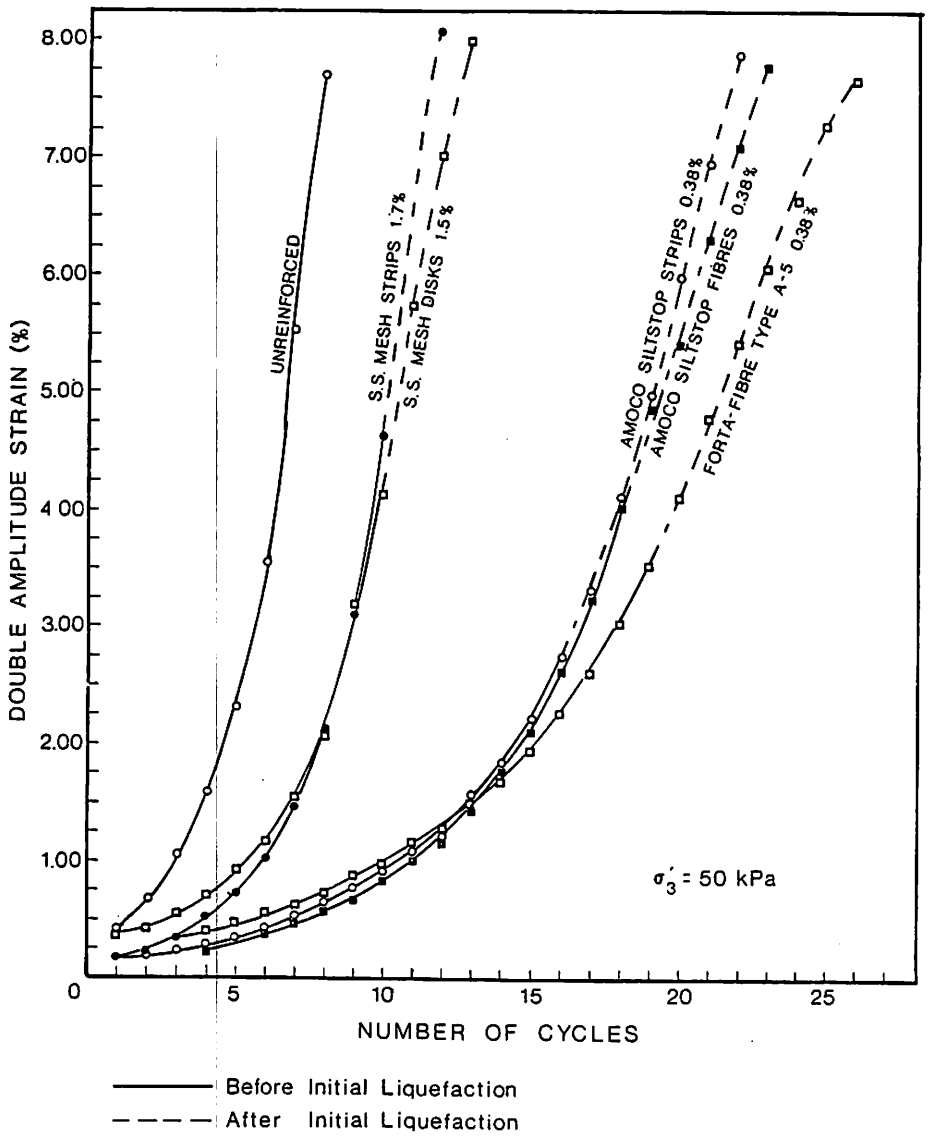

Figure 2. Comparison of strain development during cyclic loading of unreinforced specimens and specimens reinforced with various materials. The stress ratio was 0.52 in each test.

comparison between the behavior of unreinforced sand and sand reinforced with different types of reinforcement materials. Figure 2 shows a comparison between the strain developed during cyclic loading of unreinforced specimens and reinforced specimens.

On the basis of the results of the cyclic triaxial tests the following conclusions can be made:

1. Specimens reinforced with widely spaced (four layers in 18 cm.) discrete layers of polypropylene fabric exhibited no significant increase in resistance to liquefaction. Specimens reinforced with widely spaced, discrete stainless steel mesh disks exhibited some increased resistance to liquefaction at high stress ratios but no significant effect at low stress ratios.

2. Specimens reinforced with 0.38% by weight of closely spaced (15 layers in 18 cm.) discrete layers of polypropylene fabric strips exhibited some increased resistance to liquefaction at all stress ratios tested.

3. Specimens reinforced with 0.38% by weight of randomly distributed polypropylene fibers exhibited a marked increase in resistance to liquefaction.

4. The more evenly distributed a reinforcement is throughout a specimen, the more effective it is in increasing the specimen's resistance to liquefaction.

For a given stress ratio and a given number of cyclic load applications, the axial strain in reinforced specimens was lower than in unreinforced specimens. In addition, the axial strain at initial liquefaction of reinforced specimens was lower than in unreinforced specimens.

6. Beyond initial liquefaction, randomly distributed fiber reinforced specimens withstood several additional cycles at high stress ratios compared to unreinforced specimens.

The beneficial effect of inclusions on the dynamic behavior of sand is due to the continuity provided by fabric strips or fibers within the soil matrix. In a loose to medium-dense uncemented sand, much of the induced strains are permanent. With the increase of plastic strain, non-spherical particles move and rotate in order to assume the most stable positions under the condition of the applied stresses; the movement of an individual particle becomes more restricted as the number of contacts it has with other particles increases. Even when the externally applied stress system is entirely compressive, tensile strains develop within the soil mass as a result of particle movement, and soil (particularly sand) is weak in tension. If tension resistant inclusions are present within a soil, they will be strained and so develop tensile resistance. This will induce a tranfer of stress from the soil to the inclusions, thereby reducing the strains induced within the soil by the externally applied stress system, thus restricting the movement of particles. In the case of fiber inclusions, this load-transfer mechanism is essentially a result of surface friction between the fibers and the individual soil particles.

During specimen preparation, it was noted that specimens with

either strip or fiber inclusions mixed with sand required more compactive effort than the unreinforced specimens. This same mechanism by which the sand-reinforcement mixture resists compaction also acts to impede particle movement in response to externally applied stresses. If those particles in contact with a reinforcement are impeded from moving, then particles in contact with them will also be somewhat restrained. The influence of the reinforcement will extend in this fashion to a region of sand particles surrounding the reinforcement. It seems reasonable that the more evenly distributed reinforcements are throughout a specimen (or sand mass), the greater number of particles will either be in direct contact with or within the zone of influence of reinforcement, and the more effective will be the reinforcement in reducing the tendency of the particles throughout the specimen to rearrange themselves, build pore pressure, and cause liquefaction.

The restraining effects of randomly distributed reinforcements were clearly observed in terms of the strains measured during the cyclic triaxial tests. For a given stress ratio and a given number of cyclic load applications, the axial strain in reinforced specimens was lower than in unreinforced specimens. In addition, the axial strain at initial liquefaction of reinforced specimens was lower than in unreinforced specimens. Unlike sand, the fibers used in this study are elastic materials. Their inclusion within the sand specimens increased the specimen's overall elasticity by absorbing some of the strain induced within the sample in response to the cyclic loads. Between cycles when the specimens were not stressed the elastic reinforcements rebounded, thus decreasing the amount of overall plastic (permanent) strain experienced by the sand with each load cycle.

This study showed that mixing a small amount (less than 0.5% by weight) of synthetic fibers to sand can improve its resistance to liquefaction. Although the mixing process for fills placed above water is relatively easy, no simple procedure seems to be apparent at this time for the placement of fiber-reinforced fills under water.

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Aquaculture

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Genetic Engineering: Modern Technology Applied to Improvements in Molluscan Aquaculture

University of California, Santa Barbara
R/A-51
Project Initiated: October 1, 1982
Project Completed: September 30, 1985

Daniel E. Morse

Our major objective is to use techniques of genetic engineering and molecular biology to identify those hormones and mechanisms that control rapid early growth in abalones, and then based on these findings, to develop economical and practical methods to accelerate the growth of abalones and other molluscs in culture. As described below, results of this research are already being implemented in applications to improve abalone production in Japan.

We have found that exogenous insulin and growth hormone can increase the food conversion efficiency and rate of growth in young juvenile abalones (*Haliotis rufescens*) (Morse, 1981, 1984a,b, 1985; Hooker and Morse, 1985), thus suggesting that endogenous production of these hormones normally may limit and control growth. In progress reported last year, we have extracted and purified abalone insulin and quantitated the distribution of this hormone in various tissues using sensitive radioimmunoassays. Our results revealed that the highest concentrations of hormone are distributed throughout the digestive tract mucosa of the mollusc rather than being consolidated as in animals with a true pancreas. These findings confirmed and extended earlier observations of Collip (1923), DeMartinez et al. (1973), and Falkmer et al. (1973) and support the suggestion made by Falkmer et al. (1973) that insulin released by the molluscan intestinal mucosa may act directly on the digestive tract endothelium to enhance nutrient assimilation (and thus food conversion efficiency) in these species.

This past year we have purified and further characterized several enzymes and somatic proteins that we have found particularly useful for studies of the hormone-dependent regulation of growth and specific protein synthesis at the tissue and molecular levels. We have purified to homogeneity, and have extensively characterized, the enzyme glucose-6-phosphate dehydrogenase (Vivas, 1985; Vivas and Morse, in prep.). This enzyme, which is centrally important in energy metabolism, biosynthesis, and growth, has been purified over 1000-fold by cofactor affinity chromatography, gel filtration, SDS gel electrophoresis, and renaturation to yield the pure active enzyme. The purified enzyme has been characterized with respect to molecular weight, subunit structure, cofactor control of subunit and oligomeric association, isoelectric point, substrate and cofactor specificity, thermal stability, catalytic properties, and amino acid composition. The high activity and stability of this enzyme, its tissue distribution, and its ease of extensive purification by a one-step affinity chromatography procedure that we devised make this enzyme especially useful for measurements of the hormone-dependent control of muscle tissue growth.

This year we have developed improved methods for the detection and purification of the major calcium-binding peptides from molluscan shell, and we have used these methods to purify to homogeneity and characterize the six major peptides extractable from abalone shell conchiolin (Cariolou, 1985; Cariolou and Morse, in prep.). Amino acid compositional analysis and spectroscopic data indicate that the principal peptides consist largely of alternating glycyl-aspartyl sequences, thus accounting for the unusual physical and calcium-binding properties of these proteins (Cariolou and Morse, 1985). The rapid growth response and the repeating, simple structures of these peptides that we have found (compatible with block synthesis of synthetic oligonucleotide DNA probes now underway in our laboratory) make them especially useful for sensitive measures of hormone-dependent growth responses of the shell at the tissue and molecular levels.

Also this year, we have identified and purified two different molecular forms of the abalone's digestive enzyme arylsulfatase. Now reproducibly able to resolve these two forms quantitatively, we have identified significant differences between the two forms in electrical charge, molecular weight, and substrate affinities. Preliminary data indicate that the growth-dependent increase we have observed in this enzyme following metamorphosis is accounted for by differential synthesis of only one of the isozymes. Our ability to now resolve the two forms of the enzyme quantitatively thus makes analysis of the differential synthesis of these isozymes a sensitive and specific measure of digestive tissue development and growth.

In work directed at the abalone insulin gene and its expression, we have this year synthesized mixed families of more than 200 oligonucleotides, including all of the predicted 14- and 21-nucleotide sequences corresponding to the most highly conserved receptor-binding domains of the insulin molecule. In experiments using these oligonucleotides as labeled DNA probes, we have verified strong homology of the synthetic oligonucleotide families with the cloned coding sequences of the insulin genes from angler fish and rat. These probes now are being

used to quantitate insulin mRNA synthesis in the abalone intestinal mucosa and to identify those factors that regulate insulin gene expression and endogenous production of this hormone in the abalone. These insulin oligonucleotide probes are also being used to identify the cloned abalone insulin genes in recombinant DNA gene banks we have constructed using phage λ.

Although the Sea Grant-supported phase of these studies has been concluded, we have succeeded in transferring support for the continuation of this research and development to private industry. It is anticipated that this support will make it possible for us to achieve the overall project goals.

After our extensive dissemination in Japan of our experimental results, in which we demonstrated that exogenous insulin from vertebrate animals could be used to accelerate the growth of young abalone (Morse, 1981, 1984a), Japanese researchers have successfully used this basic method with salmon growth hormone to improve production of abalone (Takahashi et al., pers. commun.). Additional links in our research to organizations and activities in other Pacific rim countries have been extensive. They include collaborative research conducted at the Oyster Research Institute, Japan; collaborative research conducted at the Institute of Oceanology, People's Republic of China; collaborative research conducted at the Central Marine Fisheries Research Institute, India; activity as principal organizer, co-host, and co-editor of the Sea Grant-sponsored International Symposium on Recent Advances in Cultivation of Pacific Molluscs (at Scripps Institution of Oceanography, December 1982), organizer and moderator of a special symposium at the International Symposium on Marine Plankton, in Japan, 1984; research visits and lectures at Universities and at fisheries and aquaculture research and development centers in Japan, People's Republic of China, Thailand, India, New Zealand, Fiji, and the Cook Islands; advice and assistance to commercial aquaculture firms in the Philippines, Japan, the Republic of China, and Chile; advice to government aquaculture and marine biotechnology programs in Japan, the People's Republic of China, Republic of China, Mexico, New Zealand, and Australia; participation in the U.S. National Academy of Sciences/Republic of China Aquaculture Program; and demonstration of our research and new methods in our laboratory to visiting aquaculture scientists from Japan, the People's Republic of China, Republic of China, Australia, and Mexico.

Similar levels of cooperation also exist between our laboratory and countries in the Caribbean and in Europe.

Cooperating Organizations

Ab Lab, Inc., Port Hueneme, California
Atlantic Richfield Foundation, Los Angeles, California
Beckman City of Hope Medical Research Foundation, Duarte, California
California Abalone Association, Santa Barbara, California
Central Marine Fisheries Research Institute, Cochin, India
Central University of Venezuela, Caracas, Venezuela
Chevron USA, Inc., San Francisco, California
CNEXO Aquaculture Laboratories, Brest Cedex, France
Dynasen Mariculture, Santa Barbara, California
Genentech, Inc., South San Francisco, California
International Women's Fishing Association Scholarship Fund, Palm Beach, Florida
Iwate Prefectural Fish Farming Center, Ofunato-shi, Japan
Johns Hopkins University, Baltimore, Maryland
McNaughton Scholarship Fund, Trenton, Michigan
Miyagi Prefectural Fish Farming Center, Miyagi-shi, Japan
Oyster Research Institute, Kesen-numa, Japan
Portsmouth Polytechnic Institute, Hayling Island, Portsmouth, U.K.
Seafood Specialties, Inc., Santa Barbara, California
Tohoku Regional Fisheries Research Laboratory, Shiogama, Japan
Tohoku University, Sendai, Japan
University of California, San Francisco
University of Maryland Sea Grant College Program, University of Maryland, Adelphi and College Park, Maryland

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Lectures

Morse, A. N. C. and Morse, D. E. GABA-mimetic proteins from marine red algae. Invited Symposium Presentation, Symposium on Biomedically Active Compounds from Marine Algae, XIth International Seaweed Symposium, Qingdao, China, June 1983.

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Morse, D. E. Biochemical and genetic engineering for improved production of abalone and other valuable molluscs. Invited Symposium Presentation, International Symposium on Recent Innovations in the Cultivation of Pacific Molluscs, San Diego, California, December, 1982.

Morse, D. E. Genetic engineering in molluscs. Invited Lecture, Agouron Institute, San Diego, California, January 1983.

Morse, D. E. Biochemical control of reproduction, metamorphosis and growth in *Haliotis*. Invited Lecture, Institute of Environmental Stress, University of California, Santa Barbara, April 1983.

Morse, D. E. Marine biotechnology: New directions for research and graduate education. Invited Lecture, National Oceanic and Atmospheric Administration Sea Grant Council of Directors, and National Sea Grant Week Program, San Antonio, Texas, July 1983.

Morse, D. E. Biochemical signals, receptors and transducers controlling the settlement and metamorphosis of marine invertebrate larvae. Invited Panelist and Presentation, Session on Microbial Cues in the Development of Marine Invertebrates, at the Third International Symposium on Microbial Ecology, Michigan State University, August 1983.

Morse, D. E. Biochemical and genetic engineering in marine aquaculture: The role of modern biotechnology in the production of food from the oceans. Invited Symposium Presentation, International Marine Technology Symposium, "Oceans '83," San Francisco, California, August 1983.

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Morse, D. E. External molecular signals controlling reproduction, settlement and metamorphosis of benthic marine invertebrates. Invited Symposium Presentation, International Symposium on Life Histories of Benthic Marine Invertebrates, Aurangabad, India, January 1984.

Morse, D. E. Recent developments in mariculture, marine fouling, and bioactive substances from marine algae and bacteria. Invited Lecture, National Institute of Oceanography, India (Dona Paula), January 1984.

Morse, D. E. Prostaglandin control of reproduction in marine invertebrates. Invited Lecture, Central Marine Fisheries Research Institute, Cochin, India, February 1984.

Morse, D. E. Applications of genetic engineering for improvements in mariculture. Invited Lecture, Central Marine Fisheries Research Institute, Cochin, India, February 1984.

Morse, D. E. Characterization of biochemical inducers, larval receptors, and transducers controlling settlement and metamorphosis. Invited Lecture, Central Marine Fisheries Research Institute, Cochin, India, February 1984.

Morse, D. E. Genetic engineering for improved production of valuable marine species. Invited Presentation, California Sea Grant Aquaculture Meeting, University of California, Davis, California, April 1984.

Morse, D. E. Molecular signals and genetic engineering in marine science and technology. Invited Symposium Presentation, Marine Biotechnology Symposium, American Association for the Advancement of Science Meeting, New York, New York, May 1984.

Morse, D. E. Neurotransmitter-mimetic inducers of larval settlement and metamorphosis. Invited Symposium Presentation, Symposium on Mechanisms Controlling Recruitment of Larvae from the Plankton, International Symposium on Marine Plankton, Tokai University, Shimizu, Japan, July 1984.

Morse, D. E. Biochemical and genetic engineering for improved control of reproduction, development and growth in abalone and other molluscs. Invited Lecture, Tohoku University, Sendai, Japan, August 1984.

Morse, D. E. Marine biotechnology. Invited Address, New Horizons in Science Meeting, Council for the Advancement of Science Writing, San Francisco, October 1984.

Morse, D. E. Environmental chemical signals, larval receptors and transducers controlling substratum-specific recruitment of marine invertebrate larvae. Invited Symposium Presentation, Larval Biology Symposium, American Society of Limnology and Oceanography, San Francisco, December 1984.

Morse, D. E. Biochemical signals controlling reproduction, development and growth of marine invertebrates. Invited Presentation, U.S. National Research Council–Republic of China Workshop on Aquaculture Engineering, Honolulu, January 1985.

Morse, D. E. Molecular mechanisms controlling reproduction, development and growth in marine invertebrates. Invited Presentation, Howard Hughes Medical Institute–Woods Hole Oceanographic Institution Workshop on Marine Sciences and Biomedical Applications, Woods Hole, February 1985.

Morse, D. E. Hormone and neuropeptide control of reproduction, development and growth in abalone and other marine invertebrates: Applications of commercial significance. Invited Lecture, Genentech Corporation, San Francisco, March 1985.

Morse, D. E. Neurotransmitter-mimetic inducers and their cognate receptors controlling substratum-specific settlement and metamorphosis of marine invertebrate larvae. Invited Presentation, Larval Biology Workshop, Friday Harbor Laboratory, Washington, March 1985.

Morse, D. E. Molecular control of marine invertebrate reproduction, development and growth. Invited Symposium Lecture, BioExp 85 International Biotechnology Conference Symposium on Marine Biotechnology and Aquaculture, Boston, May 1985.

Morse, D. E. Enhanced production of food and new medical resources through marine biotechnology. Invited Symposium Lecture, Symposium on Marine Biotechnology: Prospects for the Americas. American Association for the Advancement of Science Annual Meeting, Los Angeles, May 1985.

Trapido-Rosenthal, H. and D. E. Morse. Diamino acids facilitate settlement and metamorphosis of molluscan larvae. Symposium Presentation, American Society of Zoologists Meeting, Denver, December 1984.

Yool, A. J., and D. E. Morse. Ionic control of metamorphosis in gastropod larvae. Symposium Presentation American Society of Zoologists Meeting, Denver, December 1984.

In Vitro Cultivation of Marine Crustacean and Molluscan Cells

California State University, Long Beach
R/A-56
Project Initiated: October 1, 1983
Project Completed: September 30, 1985

Laura Kingsford and Douglas W. Hill

Currently there are no known methods for isolating and identifying the etiological agents of viral diseases of marine invertebrate organisms. Thus, it is difficult, if not impossible, to diagnose any form of viral disease (acute, chronic, latent, or otherwise) in these important animals. This applies not only to infections occurring to them in their natural habitat, but also in situations where humans are able to propagate economically significant species for food. The threat of epizootics of viral diseases in these species under conditions of aquaculture is of enough importance to the future to justify considerable efforts toward the development of the necessary technology to successfully isolate and to identify specific viral agents of disease of marine invertebrates. To this general end, this research was directed. The main reason for the inability to study the viruses of these animals is that, to the present time, no one has been successful in establishing a line of cells in culture that is susceptible to any virus of these animals. The specific goal of this research was to use two model animals, edible clams (*Mercenaria mercenaria*) and the shrimp *Sicyonia ingentis*, as sources of tissues in attempts to grow populations of cells that could be used to infect with viruses from these same animal species.

The archival literature records few successful descriptions of the growth of aquatic invertebrate cells in culture. Oyster amoebocytes (Tripp et al., 1966) and cells from mantle explants (Perkins and Menzel, 1964), octopus white body cells (Necco and Martin, 1963), and marine snail cells (Ebstein et al., 1965) have been reported to have been maintained for various times but with no evidence of mitoses. Only two authors, Cecil (1969) and Phillips (1961), have reported actual growth of cells *in vitro*. No subsequent papers from these authors on this subject have been forthcoming.

With the exception of insect cells, of which at least 119 lines from 51 species have been established (Hink, 1979), the only continuous invertebrate cell line isolated and available for use is from the freshwater snail *Biomphalaria glabrata*. These cells were derived from trochophore larvae and are now stored in the American Type Culture Collection as CRL #1494 (Basch and DiConza, 1973).

The primary animal species employed in these studies was *Mercenaria mercenaria*, the cherrystone clam. It was chosen for use because of ease of availability during all times of the year and its economic importance. For additional studies, a crustacean, *Sicyonia ingentis*, a locally available shrimp was included. Adult individuals of this latter species were supplied by Dr. Donald Reish, Department of Biology, California State University, Long Beach, who collected them off the southern California coast.

Extensive studies on different tissues of the clam established that tissues from the heart yielded the most successful maintenance cultures. Other tissues were found heavily contaminated with bacteria and protozoa or yielded cells that failed to grow.

Cells from cardiac tissue were contractile, attached to culture surfaces, and spread out from the periphery of tissue fragments. Control studies established that a basal medium of artificial seawater, Eagle's minimum essential medium (MEM), calf serum, and antibiotics supported maintenance of cells for prolonged periods of time when incubated at 20°C, the optimal temperature.

Dissociation of the cardiac tissues into clumps of cells was best accomplished by mechanical means. Trypsin, ethylene diamine tetraacetic acid disruption was noted to be highly damaging to these cells.

Other variables concerning the composition of the culture medium were examined. The optimal conditions noted were 10% sterile calf serum, use of glucose rather than trehalose, a pH of approximately 8.3, and an ionic environment equivalent to that of the basal medium with 60% artificial seawater, 30% MEM, 10% calf serum, and 0.05 *M* sodium bicarbonate.

Normal polystyrene microtiter plates were used in all of the above studies for attempts to grow clam cardiac cells. Other surfaces were compared to it as candidates showing possible improved growth. Fibronectin and concanavalin A treated polystyrene exhibited significant improvement in attachment and spreading of fibroblasts. However, polylysine and collagen treatment showed no improvement in growth.

Numerous growth factors and/or hormones were added to the basal medium in attempts to stimulate the clam cardiac cells to grow. Fibroblast growth factor at concentrations between 10^{-12} to 10^{-14} *M* significantly enhanced the ability of the basal medium to maintain morphologically intact cells as did ecdysterone at approximately 10^{-12} to 10^{-14} *M* concentrations. Epidermal growth factor, γ-amino butyric acid, endothelial growth factor, and insulin at any of the concentrations employed were found to be without any discernible effects. The concentration ranges

used were those found to be effective in mammalian cell systems. Actually, insulin at concentrations found to stimulate growth of mammalian cells (Barnes and Sato, 1980) appeared to be toxic for clam cardiac cells.

No convincing evidence has been collected to suggest that clam cardiac cells actually propagated under the above-described conditions. At best, improvement in maintenance of these cells in the basal medium containing mammalian fibroblast growth factor or ecdysterone was observed. Fibronectin-treated polystyrene afforded improved attachment and spreading of the cells.

Cells isolated from shrimp body muscle and heart tissues were isolated and plated out in the basal medium, with added fibroblast growth factor and/or ecdysterone at concentrations enhancing survival of the clam cardiac cells. These cell preparations in these media were grown on standard polystyrene surfaces and the same coated with two different concentrations of mammalian fibronectin or poly-L-lysine. Incubation at 20°C for prolonged times and observations yielded evidence for survival of only cardiac cells.

In frustration and in attempts to determine hormone requirements for growth of marine invertebrate cells, assays for identifying hormone receptors on cell surfaces were investigated. Insulin, a hormone required for just about every mammalian cell (Barnes and Sato, 1980) was chosen as the first candidate. Plasma cell membranes of clam tissues were solubilized, and purified proteins were electrophoresed and immunoblotted. Three proteins were detected with molecular weights corresponding to mammalian insulin receptor subunits. None of these proteins bound to insulin when purified by affinity chromatography. This suggests that clam cardiac cells may have insulin receptors but that they are different from mammalian ones. They may have a low binding affinity for porcine insulin, the form used in all of these studies. These investigations are ongoing in attempts to identify receptors for other hormones or growth factors on marine invertebrate cells.

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Nutrient Uptake by Fish Intestine

Department of Physiology
University of California Medical School, Los Angeles
R/A-57
Project Initiated: October 1, 1983
Project Completed: September 30, 1985

Jared M. Diamond, Randal Buddington, and Cathryn Saul-Conn

The goal of our project is to obtain information about nutrient absorption in fish intestine that is essential for the rational design of fish diets. Feeds are a major expense in fish production because fish need balanced high-protein diets for maximal growth. These requirements are usually met by fish meal itself, which is expensive. Attempts to produce cheaper diets by incorporating plant protein, often with various supplements, have generally yielded lower growth rates and have not been cost-effective. Too little is known about nutrient absorption by fish intestine to guide the design of more cost-effective diets. We know little about the mechanisms of nutrient absorption by fish intestine and how they change with diet, age, and reproductive status. To obtain such understanding and to apply it to the design of improved diets are our overall goals.

We continued a study this year that we began in the previous year, which was to examine differences in intestinal nutrient absorption among fish species with different natural diets. We also examined whether there are species differences that are genetically programmed instead of being immediately triggered by the diet of the moment. To do this, we compared nine species, all maintained in our laboratory on the same fish feed. The species ranged from carnivores through omnivores to herbivores. We measured the total capacity of the intestine for uptake of the carbohydrate breakdown product glucose and of the amino acid proline as a protein breakdown product. The ratio of proline to glucose absorption increased with protein content in the fish's natural diet. Thus, carnivores are preprogrammed to specialize in the absorption of amino acids, while herbivores are preprogrammed to specialize in absorption of carbohydrates. In short, each fish species is genetically adapted to its natural diet.

Next, we examined whether fish intestine could adapt to a dietary change within the fish's lifetime. We maintained populations of trout and carp on a high-protein carbohydrate-free diet and also on a high-carbohydrate medium-protein diet. Trout are carnivores, and carp are herbivores or omnivores. Carp did well on both diets and experienced a relative increase in glucose absorption on the high-carbohydrate diet. However, trout did less well on the high-carbohydrate diet than on the high-protein diet, and on the former diet their absorption of both sugars and amino acids declined. Thus, the herbivore is better able to adapt phenotypically to dietary carbohydrate than is the carnivore.

Developmental changes in intestinal function with age and reproductive status are likely to be important for fish. We have studied a Californian saltwater species, the monkey-faced prickleback, of which young individuals are carnivores but which become increasingly herbivorous as they grow. We found that the ratio of proline to glucose absorption decreased from smaller to larger fish, although all the fish were being maintained in our laboratory on the same fish meal. Thus, the intestine is preprogrammed to develop with age in a way that matches it to the change in natural diet that the fish would normally be encountering at that body size.

We recently initiated a study to examine how the rate of appearance of amino acids in plasma following a meal is affected by the dietary form in which protein is administered. Our preliminary results show that diets of protein, protein-hydrolysate, and three amino acids exhibit major differences in the plasma time course of nutrient buildup.

Cooperating Organizations

California State Department of Fish and Game
Coachella Valley Water District
Fish Breeders
The Fishery
Institute of Marine Biochemistry, Aberdeen Scotland
Pacific Aqua Farms
Star Milling Company
University of Barcelona, Spain
White Water Trout Company

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Evaluation of Protective Antigens of *Aeromonas salmonicida*

University of California, Davis
R/A-58
Project Initiated: October 1, 1983
Project Completed: June 30, 1986

Ronald P. Hedrick

The overall objective of this project was to elucidate the protective antigens of the bacterium *Aeromonas salmonicida*, the causative agent of furunculosis in salmonid fishes. This was approached by purifying suspected virulence factors or other factors assumed to be associated with protection in previous immunization studies. The three principal antigens of the bacterium examined were the A protein, an outer layer found mostly on virulent strains (McCarthy et al., 1983), the endotoxin or lipopolysaccharide (LPS), and an extracellular product shown by Cypriano (1982) to have potential as a protective immunogen.

Each of these three antigens has been purified from two known virulent strains of *A. salmonicida* as (AS-1R and 3.101) and two avirulent strains (AS-1S and 395). The purified antigens were used to produce specific antisera in both rainbow trout (*Salmo gairdneri*) and white rabbits. The antiserum was then examined for its specific response to the purified antigen by gel diffusion and immunoelectrophoresis. The rabbit and trout antisera will now be used to passively immunize chinook salmon (*Oncorhynchus tshawytscha* that will subsequently be challenged with virulent strains of *A. salmonicida*.

During the course of these studies a variant (3.101-2) of the avirulent strain 3.101-1 (obtained from the originally virulent 3.101 strain) was obtained. This particular variant was of interest because it was derived from a smooth (no A layer) strain but itself possessed an A layer and was avirulent. Since the A layer is believed to be involved in virulence (Udey and Fryer, 1978) further studies were conducted on this isolate. Electrophoretic comparisons between 3.101-2 and its parent isolate showed they shared an A protein of identical molecular weight. In addition, the variant autoaggregated and formed rough colonies on agar surfaces. The variant was tested in rainbow trout and shown to be avirulent by the inability to recover bacteria from injected fish and the absence of mortality. In contrast, bacteria were recovered from all fish injected with equal numbers (10^4 colony forming units) of strain AS1R, and all fish died within 96 hours.

Preliminary trials using strain 3.101-2 as an immunogen indicate that this isolate may be useful in bacterins designed to reduce *A. salmonicida* infections in salmonid fishes.

Cooperating Organizations
California Department of Fish and Game

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Fisheries

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The Effects of Climate and Weather on Albacore Migration and Distribution in the Northeastern Pacific

University of California, San Diego
R/F–86
Project Initiated: October 1, 1983
Project Completed: September 30, 1986

Reuben Lasker and Jan Svejkovsky

The overall objective of this project is to determine the influence of climatic and weather fluctuations on the migration and distribution of albacore tuna along the North American coast. This is to be accomplished by utilizing satellite remotely sensed data to monitor surface thermal patterns and by using surface wind stress estimates to monitor the variability in atmospheric forcing in relation to albacore distributions.

The goals for the 1985–86 funding period, which represented the third and final year of the project, were (1) to complete the processing and analyses resulting in a workable model, (2) to test the conclusions of the analyses on new fishery data made available during the project, and (3) to disseminate the results of the study.

At the time of this writing, model development using the fishery, satellite and wind data sets has been completed. The input data covered the U.S. albacore fishing seasons between 1980 and 1983. Data from 1984 was to be used to test the model by utilizing satellite image analysis results and wind measurements to make hindsight predictions of albacore distribution patterns and to compare them with actual patterns reflected in commercial catch records. This final comparison is also near completion. The results of the study were made available to the albacore research group of the Southwest Fisheries Center of the National Marine Fisheries Service. The results will also be published in two scientific papers which are under preparation and as part of a book, *Marine Organisms as Indicators*, to be published by Springer-Verlag (New York).

Additionally, an efficient satellite image analysis technique for measuring sea surface flow was developed to aid image analyses during this project. A paper describing the technique and determinations of its accuracy has been submitted to the *Journal of Geophysical Research* and is presently under review (Svejkovsky, submitted).

As is described in previous reports from this project, specially processed images of sea surface temperature from the Advanced Very High Resolution Radiometer (AVHRR) were used to monitor the variability of thermal/color frontal patterns in the ocean's upper layer. The variability was quantified through the calculation of spatial correlation coefficients for one degree latitude/longitude areas from images separated by a six- to eight-day interval. The distribution patterns of albacore tuna were estimated from commercial catch records compiled by the Southwest Fisheries Center and standardized to the same spatial and temporal scales as the satellite data.

A strong relationship was found between the degree of localized frontal structure change and albacore availability. In regions with high correlation values, indicating frontal structure persistence, fishing success generally remained steady or improved. Conversely, when a region showed low correlations, indicating rapidly changing frontal structure, catch rates declined significantly. These findings were interpreted as resulting from albacore becoming aggregated in regions with an established, stable frontal regime and dispersing out of regions where the frontal patterns become disturbed.

During this project a technique was developed to estimate upper layer flow rates by tracking submesoscale thermal features in AVHRR images recorded 12 to 24 hours apart. This procedure allows the estimation of surface currents over large areas even in the absence of *in-situ* measurements. Current fields obtained in this way confirmed published results from other investigations which showed high flow rates to exist often along the frontal boundaries, especially on their cold water side. The gradient currents make the boundaries into zones of high shear. However, under certain conditions, these zones attain a relative geostrophic balance and persist for weeks with little change in the overall frontal pattern and intensity. This is especially true under low wind conditions. Studies in the California Current have shown that high winds or storms with winds in excess of 10 cm/s generate drift currents that overpower the gradient currents and rapidly alter the established frontal regime.

The inclusion of daily wind measurements in our analysis showed that stable conditions associated with high fishing success existed predominantly during calm weather periods. Conversely, large frontal pattern changes and decreases in fishing success were often preceded by periods of high winds, lasting for two days or more. The lag period between the high wind event and the destruction of the established frontal pattern averaged five days.

Obviously, the relationship between wind forcing and frontal pattern variability is a very complex one. The extent to which a one degree latitude/longitude area becomes affected by a high wind period varies with the local conditions, geostrophic field, and

current dynamics. In comparison, the relationship between albacore aggregation and frontal pattern changes appears to be much more direct. Atmospheric forcing is therefore a less reliable indicator of the short-term variability in albacore distribution than is the satellite-image-derived correlation analysis. However, the five-day lag in ocean response to atmospheric effects offers the possibility of predicting potential large changes in frontal structure and hence also in albacore distribution. Based on this idea we developed a prototype forecasting model for predicting shifts in albacore distribution patterns based on catch reports, atmospheric forcing analyses and the satellite-derived frontal variability in a given area at a given time.

The model uses recent catch reports and satellite-derived correlation analysis to establish the existing fish distribution and frontal pattern dynamics on a one degree latitude/longitude scale. An atmospheric forcing index computed from recent surface wind data is then used to estimate the potential for frontal structure change in each area in the next seven days. Areas that show an established frontal structure, have positive catch reports, and were not affected by high winds are deemed prime aggregation areas. On the other hand, regions with an established frontal pattern that experienced high winds are expected to show a rapid decline in albacore availability. Likewise, regions experiencing initial high frontal variability (low correlation values) will have poor fishing success.

As was mentioned above, this forecasting technique is being verified by making hindsight weekly predictions during the 1984 season and comparing the results with actual catch statistics compiled by the Southwest Fisheries Center. The results appear very encouraging. It must be noted, however, that the technique is based on the albacore's apparent response to environmental changes as measured by the parameters used. Therefore, the model does not predict the existence of albacore in certain localities. Rather, it predicts the response of the fish (continued aggregation or dispersal) to environmental variability in regions where albacore presence has been verified by fishing vessels.

The initial presence of albacore in a region is governed by the seasonal migration cycle, water temperature, prey availability, and many unknown factors. Broad climatic events, e.g., the 1983 El Niño, significantly affect the timing of albacore's initial entry into U.S. coastal waters. Severe weather at the end of the season (September and October) may cause the fish to leave the U.S. waters early. While within the California Current system, however, albacore respond to the studied relationships quite consistently. We found no significant differences in albacore response, as studied in this project, between the warmer 1983 and the preceding years.

Cooperating Organizations

National Marine Fisheries Service, Southwest Fisheries Center, La Jolla

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Publications

Svejkovsky, J. Submitted. Sea surface flow estimation from AVHRR and CZCS satellite imagery; A verification study. *J. Geophys. Res.*

Contributions of Coho and Chinook Spawning Populations to Mixed Fisheries: A Management Study

University of California, Davis
R/F-87
Project Initiated: October 1, 1983
Project Completed: September 30, 1986

Graham A. E. Gall

The primary goals of the project were to characterize the genetic structure of coho and chinook populations, to assess genetic differentiation among populations, and to develop a set of baseline data consistent with data being collected by others for purposes of analyzing mixed ocean fishery harvests. The long-term goal is to provide a methodology for the analysis of mixed fishery harvests to provide a new tool for managing the fishery that would permit optimal harvest while conserving the resource. The achievement of these goals required extensive sampling of hatchery and wild salmon populations, augmentation and refinement of appropriate electrophoretic techniques, and the coordinated collection of data on the genetic make-up of all source populations that could potentially contribute to the mixed fishery. The accomplishments of the project were greatly enhanced by the cooperation and participation of a number of agencies, particularly direct financial support from the California Department of Fish and Game.

Chinook Salmon

Analysis of chinook salmon now consists of starch-gel electrophoretic evaluation of alleles at 110 genetic loci. At least 26 of these loci will be effective in the analysis of chinook mixed fishery programs using only muscle and eye tissue. A major mixed fishery program has been initiated, under separate funding, to assess the contributions of three California river systems, Smith, Klamath and Eel, to the chinook harvest off California and Oregon.

Gene frequencies have been calculated for 66 loci from 24 populations of chinook salmon. Average heterozygosities calculated from the gene-frequency data ranged from a low value of 0.005 for Bogus Creek to a high of 0.121 for Rowdy Creek Hatchery. However, we have analyzed a limited number of loci for some populations so the results summarized here are biased due to incomplete data for some populations.

Indices of genetic similarities (Nei, 1972) were calculated from the gene-frequency data and used to assess population structure. There was clear evidence of a geographical component to the genetic clustering of chinook salmon populations. There appears to be a northern cluster of populations from the Klamath/Shasta drainage that includes Irongate hatchery, Scott River, Shasta River, and Bogus Creek. Furthermore, a southern cluster of chinook populations from the Sacramento/San Joaquin drainage has emerged. This group includes the Tuolumne River, Feather River hatchery, Merced River, Yuba River, Coon Creek, and the Sacramento River late fall run.

There were a few populations that appeared to be outliers or in inappropriate clusters. For example, Battle Creek and Coleman late fall-run fish from the Sacramento River currently cluster with the northern group. This result could be an artifact of the incomplete data set analyzed to date. However, there have been numerous transfers of fish among hatcheries and from one drainage to another, so many of the apparent inconsistencies in clustering may be resolved when complete hatchery records are available.

The final resolution of genetic similarities among chinook populations will be further enhanced by inclusion of a larger number of loci now available through the cooperative efforts of our own laboratory, the National Marine Fisheries Service Manchester Laboratory, and the Washington Department of Fisheries Laboratory. The analysis of coastwide distributions of chinook salmon will also be possible as a result of the cooperative effort.

Coho Salmon

Extensive analyses have been completed for a total of 60 loci identified for 52 coho populations. Forty-six of the loci are disomic while the other 14 consist of 7 duplicated, pseudo-tetrasomic loci. These duplicated loci have been scored as single entities so the number of loci "recorded" is 53. In total, 144 alleles have been observed at the 53 loci. Only seven loci were monomorphic, although 13 possessed only rare variant alleles at frequencies of 0.01 or less. Average heterozygosity estimates ranged from 0.059 to 0.137 with an average of 0.077. These results represent almost a doubling of the number of polymorphic loci reported in 1983-84.

For the 46 polymorphic loci discovered, three to five alleles were observed at 33 loci while two have been found at each of the remaining 13 loci. The association between number of alleles at a locus and estimated within-population heterozygosity was not consistent for all loci. Generally, those loci possessing only two alleles expressed the lowest levels of heterozygosity. However, there were examples of loci (Gap-2, Ldh-5) with three or four alleles which showed low levels of heterozygosity. The lack of a strong association indicates that at some loci most variant alleles occur at low frequency, while at other loci (Est-4,

lcdh-4) all alleles tend to occur at intermediate frequencies.

Gene frequencies were also calculated for 51 structural gene loci for populations of coho salmon. Average heterozygosities ranged from a low of 0.052 for fish from the Irongate hatchery and Redwood Creek to a high value of 0.133 for fish from Big Salmon Creek. Only 4 fish were examined from Big Salmon Creek; therefore, this estimate may change when more fish are analyzed.

Partitioning of total heterozygosity showed that the genetic variation attributable to diversity among populations averaged about 23%; that is, 77% of the genetic variation observed was due to differences among fish within populations. The average genetic similarity index for all pairwise comparisons among populations was 0.976, while the average similarity between individual pairs of populations ranged from 0.921 to 0.999.

A cluster analysis of coho populations based on genetic similarity indices revealed that, although all the populations have a high degree of genetic similarity, there is a geographic component to the clustering of populations. There was a distinct cluster of northern populations that included the Klamath River, Trinity Hatchery, Prairie Creek and the Smith River, and a more southern cluster that included Pudding Creek, Caspar Creek, Two Log Creek, Abion River, and Kass Creek. As with the chinook populations, there were populations of coho that do not fit into the geographic clustering pattern, such as Bogus, Flynn, and Big Salmon Creeks. Hatchery records may also provide information on coho transplanting.

Cooperating Organizations

California Department of Fish and Game
National Marine Fisheries Service, Manchester Laboratory
Oregon Department of Fish and Wildlife
Washington Department of Fisheries

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Establishment of Parameters Critical to Sturgeon Management in the Pacific Northwest

University of California, Davis
R/F-90
Project Initiated: October 1, 1983
Project Completed: September 30, 1986

Serge Doroshov, Graham Gall, and Richard Swallow

Reproductive biology of sturgeon is a key to wild stock management and domestication in aquaculture. In this study we examined reproductive conditions of wild and domestically raised white sturgeon (*Acipenser transmontanus*) from San Francisco Bay.

Wild Fish

Three discrete stages of gonadal development were found in adult fish (>90 cm fork length) during the sampling (winter) season: (1) refractory—previtellogenic follicles in females, undifferentiated cysts with only gonial cells in males; (2) active—vitellogenesis in females, spermatogenesis in males (spermatocytes in various stages of meiosis); (3) ripe—polarization of pigmented eggs in females, spermiogenesis or ripe spermatozoa in males. Based on observations of domestic broodstock and hatchery spawned wild fish, we concluded that stage 1 will be recruited to the breeding population after at least two years, stage 2 will breed the following year, and stage 3 is a current year broodstock. We examined gonadal tissues of 773 fish sampled during six consecutive winters (Table 1). More than half the sampled fish were females stage 1 (32%) and males stage 2 (28%). The breeding group, stage 3, was composed of a small number of females (9%) and approximately twice as many males (20%). The ratio of females to males was 1:1 in all adult stock and 1:2 in the breeding group, reflecting faster recruitment of males. Maturity was dependent on body size (Figure 1a). Reproductively active (stages 2 and 3) males dominated in adult male stock, particularly in 120-160 cm classes. Reproductively inactive (stage 1) females compose the majority of stock in all size classes (Figure 1b); maturing and ripe fish were mainly in the 160-180 cm classes. We assume that the reproductive potential of relatively small sturgeon is low, since only a small fraction of these adult fish are recruited for annual spawning.

Maturation of wild females was also evaluated by measuring concentrations of estrogen and vitellogenin (alkali-labile phosphorprotein, ALPP) in plasma. In stage 1, concentrations of estrogen and ALPP were low (< 0.25 ng/ml and 4.2 + 0.3 mg/ml, respectively). In stages 2 and 3, they rose to 3.68 + 0.62 ng/ml and 24.2 + 4.2 mg/ml. In gravid females prior to spawning, plasma concentrations of both sex steroid and yolk precursor significantly decreased. Thus, synthesis of vitellogenin in sturgeon (as in other fish and many vertebrate animals) appears to be controlled or at least stimulated by estrogen.

Domestic Broodstocks

Small white sturgeon colonies (35 to 150 each), born at the UCD hatchery in 1980-1984, were raised in freshwater tanks at ambient temperature and photoperiod. Fish were fed a commercial trout diet and sampled for reproductive conditions and body size. Results obtained at the last sampling (fall of 1986) are shown in Table 2. Data on wild fish aged by examination of fin sections are used for comparison. Captive males matured at age 3 years and were smaller in body size, compared with the wild stock. More than 80% of the captive males matured every year, whereas wild males appear to have a biennial cycle. Captive females had previtellogenic ovaries and low plasma concentrations of estrogen and ALPP. No vitellogenesis was observed at 3 to 6 years of age and 8 to 20 kg in mean body weight. Some wild females were ripe at age 12 to 16 years with a mean body weight of 16

Table 1. Distribution of Different Maturity Stages in the Adult Stock of San Francisco Bay*

	Females			Males			
Stages	1	2	3	1	2	3	(n)
Years							
1979-80	32	10	7	7	34	10	102
1980-81	28	6	10	0	33	23	91
1981-82	42	4	10	2	25	17	261
1982-83	13	5	7	5	45	25	99
1983-84	27	8	13	2	13	37	60
1984-85	51	6	4	11	20	8	160
Mean	32	6	9	5	28	20	N=773
CL (95%)	18-46	4-9	5-12	0-8	16-41	9-31	

*(percent of annual sample, n). Gonadal stages: 1—refractory; 2—vitellogenesis/spermatogenesis; 3—ripe.

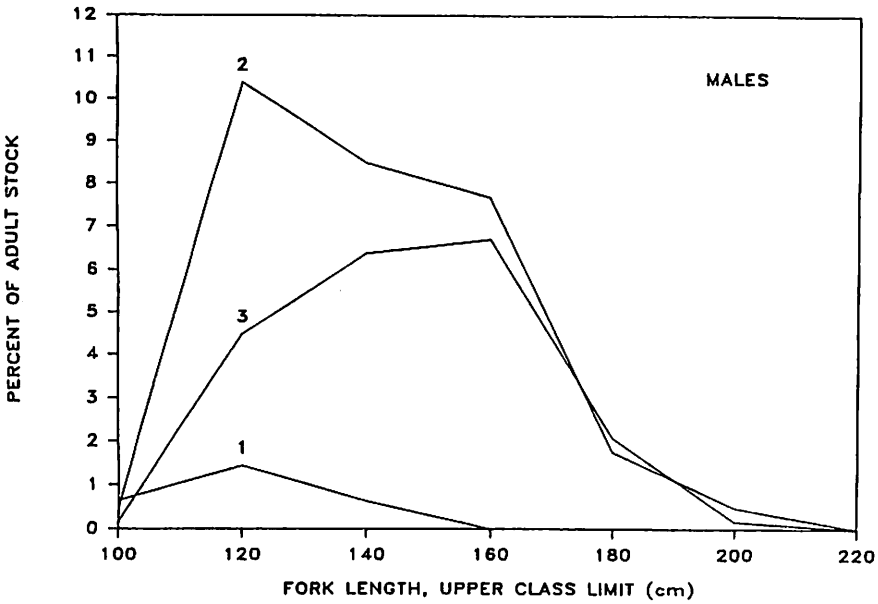

Figure 1a. Size distributions of males in adult stocks of white sturgeon at different stages of maturity (1—refractory gonads; 2—active gametogenesis; 3—ripe fish).

kg. However, the majority of ripe wild females were in age groups 18 to 26 years with body weights ranging from 25 to 45 kg. It is not clear yet, whether the observed lack of vitellogenesis in captive females is an abnormal phenomenon caused by the artificial environment. It is possible that the onset of vitellogenesis requires a certain genetically determined threshold body size (our captive colonies originated from only a few parents).

Ripe captive males are now used in commercial aquaculture for sturgeon fingerling production. We evaluated their reproductive performance by comparing five consecutive hormonally induced spermiations of five captive and five wild males. We then compared fertility and performance of their progenies, resulting from the crosses of each male to each of the 5 wild females. Data were analyzed to fit the model Yijk = u + Type + Male j(i) + Block + Type x Block + Error, where Type was domestic or wild male, Male j(i) was the random effect of either male type and Block was the random effect of females. The only significant effect was Block. No significant differences in performance were found between domestic and wild males. For domestic and wild males, mean fertility was 77% and 73%, respectively; hatchability was 54% and 51%; larval survival to metamorphosis 32% and 29%; and individual weight of fry at metamorphosis was 1.45 g and 1.33 g. In addition, there were no significant differences between the two types of males in terms of semen density or spermatozoa motility.

Hatchery Studies

We established standard tests to predict ovulatory response of wild fish to exogenous hormonal stimulation. Samples of the ovarian follicles are removed by catheter and incubated in culture medium with 10 µg/ml progesterone. Eggs are boiled and sectioned to examine the position of germinal vesicles in freshly collected follicles and are compared with the incidence of germinal vesicle breakdown in those eggs exposed to progesterone. Estimates of germinal vesicle position anticipated egg quality (fertility and viability), while hormonally induced egg maturation is a predictor of ovulatory response.

The "imprinting" effect of live diets on hatchery-raised juveniles was experimentally confirmed. Survival and growth were significantly reduced following a switch from a more natural diet (Tubifex worms) to a prepared semi-moist diet (Biodiet), whereas growth was improved and survival was improved or unaffected following the reciprocal diet switch. This suggests that progenies of wild fish are more "prepared" physiologically and anatomically to accept and assimilate the more natural diet during the early life stages and that they establish strong

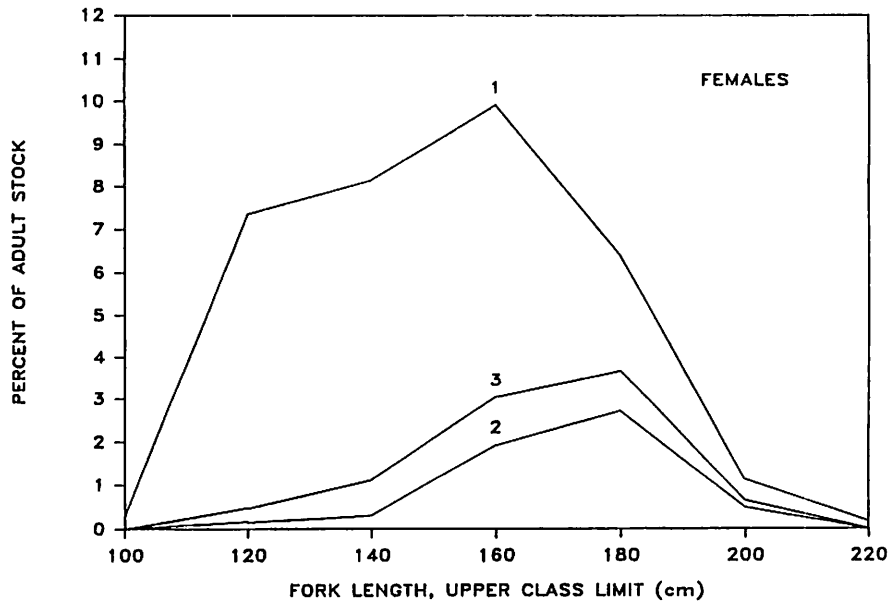

Figure 1b. Size distributions of females in adult stocks of white sturgeon at different stages of maturity (1—refractory gonads; 2—active gametogenesis; 3—ripe fish).

preference to these diets associated with specific attractants (most probably, scent). Knowledge of these factors will be needed for the improvement of existing larval diets.

Cooperating Organizations
California Aquaculture Association
California Department of Fish and Game
Center for Great Lakes Studies, University of Wisconsin, Milwaukee

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Table 2. Sexual Maturation in Domestic and Wild Broodstocks

	Domestic				Wild				
Age (years)	3	4	5	6	8–12	12–16	16–20	20–24	24–28
Females									
Ripe (%)	0	0	0	0	0	12	31	57	50
W, ripe (kg)	–	–	–	–	–	16	25	36	44
W, immat (kg)	8	9	12	20	8	13	21	31	44
Sample (n)	11	38	20	20	4	8	16	30	14
Males									
Ripe (%)	95	79	87	93	0	11	33	22	–
W, ripe (kg)	7	9	10	14	–	15	33	37	–
W, immat (kg)	7	8	10	12	8	14	27	28	–
Sample (n)	20	48	15	15	5	18	6	18	–

Assessment of the Impact of the California Sea Lion and the Northern Elephant Seal on Commercial Fisheries

University of California, Santa Cruz
R/F-92
Project Initiated: October 1, 1983
Project Completed: September 30, 1986

Daniel P. Costa

Marine mammals have long been suspected of having an adverse impact on important commercial fisheries such as salmon, herring, and squid. This project is directly assessing the impact of the most abundant pinniped of the California Coast, the California sea lion, on commercial fisheries by quantitative measurement of its prey consumption, and it is assessing the potential conflict of the northern elephant seal by quantifying its foraging habitat.

This is being accomplished with a combination of field techniques utilizing radioisotopic tracer methods and time–depth recorders coupled with validation measurements carried out in the laboratory. The radioisotopic tracer techniques employed in this study measure the free-ranging energy expenditure and the rate of prey consumption of the California sea lion, and recoverable time–depth recorders assess the foraging habitat and behavior of the northern elephant seal. These data have been integrated into the research program of the National Marine Fisheries Service, which is investigating the fisheries interactions and forage requirements of California coastal marine mammals.

Laboratory Measurements

Prey samples collected in the Southern California Bight were analyzed for water and energy content using standard techniques (Paine, 1971; Leith, 1975). Of these, Pacific mackerel, *Scomber japonicus*, was the most energy dense, followed by anchovy, *Engraulidae* sp., squid, *Loligo opalescens*, and red crab, *Pleuroncodes planipes* (Table 1).

In 1983-84 a series of laboratory measurements were begun to validate the methods for measuring food and energy intake in free-ranging sea lions. These experiments were continued in 1984-85. Validation was carried out by feeding captive sea lions weighed aliquots of food for a period of six days while simultaneous estimates of food intake and metabolism were made with the isotopic tracer techniques used in the field. In the first study the animals (two males and two females) were fed a diet of herring, and measurements of both water influx and metabolism were made. In the second study animals were fed squid, and only water flux measurements made. In the first study sea lions were not fed sufficient food to supply their energy requirements. This resulted in a small but important utilization of stored body fat, which caused a marked underestimate in metabolism that precluded validation of the doubly-labeled water methodology.

Table 1. Results of the Analysis for Water and Caloric Density of Four Common Prey Items of Sea Lions Collected in Southern California*

	Water Content	Energy Content	
Prey Item	ml/g wet	kcal/g dry	kcal/g wet
Red Crab	19.52 1.70	4.090 0.324	0.797 0.084
Squid	74.26 0.41	5.111 0.065	1.316 0.20
Anchovy	74.11 2.09	5.093 0.307	1.325 0.176
Mackerel	71.67 2.17	5.552 2.30	1.549 0.221

*Mean values are given first, followed by one standard deviation.

These studies also determined the accuracy of estimating food intake from water influx measurements. Food intake estimates are within 10.2% of herring and 1.2% of squid intake (Table 2). Food intake estimated from water influx is overestimated and may vary as a function of diet, with an error between 1 and 10%.

Measurements of the metabolic efficiency of sea lions were extended during 1984-85 to include diets of herring, anchovy, squid and mackerel. Measurements were completed on six captive sea lions (four fed herring and two other diets). Metabolic efficiency was measured by placing the animals in a metabolic cage, feeding them

Table 2. Results of the Validation Study for Estimating Food Intake on a Squid Diet from Dietary and Water Influx Data

	Mass	Water Influx	Food Intake		Energy Intake		Error
Animal	kg	ml/kg-day	Predicted g/kg-day	Actual g/kg-day	Predicted kcal/kg-day	Actual kcal/kg-day	Percent
Bill	37.4	92.3	102	94.6	111	103	7.8
Dick	30.3	110	121	125	132	136	-3.2
Judy	45.5	92.3	79.9	80.7	86.8	87.7	-1.0
Becky	31.8	96.1	106	105	96.1	114	1.0
Mean	36.3	97.7	102	101	115	110	1.2

known quantities of prey and collecting all excrement for a three-day interval. This includes urinary and fecal energy loss. The energy content of the prey and excrement was measured and the metabolic efficiency calculated, using the equation: ME = (food energy intake minus excreted energy) divided by food energy. Metabolic efficiencies range from a low of 78.3% on a diet of squid to a high of over 91% for a diet of anchovy or mackerel (Table 3).

Table 3. Average Metabolic Efficiency for Each of Four Sea Lion Prey Items*

Prey Item	Metabolic Efficiency Percent
Squid (2)	78.3 (76.1–80.5)
Herring (8)	88.2 (85.7–91.5)
Mackerel (2)	91.4 (91.1–91.6)
Anchovy (3)	91.6 (88.7–94.7)

*The number in parenthesis below the prey item is the number of experiments. The range is presented in parenthesis below the average metabolic efficiency.

Field Studies: Sea Lion Foraging Energetics

Measurements of food and energy consumption of wild sea lions were conducted during July 1983 and 1984. Studies were completed on 12 lactating female sea lions foraging between onshore nursing periods on San Miguel Island. Food intake was estimated by measuring the animals' field water influx using the tritiated water method (Nagy, 1975). Free-ranging metabolism was measured in two of the 1983 and all of the 1984 sea lions using the oxygen-18 doubly-labeled water method (Lifson and McClintock, 1966; Nagy, 1980; Schoeller and van Santen, 1982). Estimates of prey intake were converted from water flux using values for the water and energy content of squid in Table 1 and assimilation efficiency in Table 3.

Field measurements were completed as follows: lactating sea lions were captured and given an intraperitoneal injection of 3mCi tritiated water contained in 3 cm^3 sterile saline and 10 cm^3 oxygen-18 water just prior to departure from the rookery to forage. Three hours after injection a final blood sample and body weight were taken. The subjects were then released and, upon their return from feeding, were recaptured, bled, and a final body weight taken. Radiotransmitters attached to the sea lions were used to aid in resighting.

Comparisons of at-sea metabolism, food intake, and foraging efficiency between seasons will help to explain how these animals compensate for reductions in food availability. For example, it has been proposed that there is a body composition set point that regulates the metabolic intensity and duration of the foraging bout (Costa et al., 1985; Gentry et al., 1986). For example, female sea lions expended more energy foraging during the 1983 El Niño event than animals during the summer of 1984 (Tables 4, 5). In 1983 lactating sea lions consumed an average of 11.6% of their body mass per day while foraging compared to 10.4% for animals in 1984. In 1983 sea lions expended 152 kcal/kg-day to obtain prey, compared to 112 kcal/kg-day in 1984. These changes in foraging efficiency were similar to those observed for Antarctic and Northern fur seals foraging during poor seasons and good seasons (Costa et al., 1985)(Figure 1). Similar results on Antarctic fur seal show that while food was scarce during 1983-84 the rate of mass increase was significantly less than in 1984-85; however, the rate of energy consumption was not different (Figure 1). These data indicate that females do not increase the intensity of their foraging effort, but rather compensate for reduced prey intake (as evidenced by a lower mass gain) by increasing the foraging trip duration. This further suggests that they forage until a body mass set point is achieved.

Table 4. Results from Field Measurements Carried out on San Miguel Island July 1983*

	Mass kg	CO_2 Production ml/g-hr	Metabolism kcal/kg-day	Water Influx ml/kg-day	Prey Intake % Body Mass per day	Assimilated Prey Energy kcal/kg-day
Female #15 7/15–23/83	84.4	.977	142	117	13.3	154-195
Female #18 7/17–27/83	72.5	1.11	161	97	11.0	127-161
Female #19 7/17/83	88.5	–	–	–	–	–
Female #23 7/20–25/83	72.3	–	–	71	8.1	93-118
Female #24 7/20–27/83	71	–	–	117	13.3	154-195
Female #25 7/20–26/83	75.9	–	–	109	12.4	143-181
Mean	77.4	1.04	152	102	11.6	134-170
s.d.	7.3	0.09	13	19	2.2	26-32

*Prey intake as estimated from water influx is given as percent of the sea lion's body mass per day on a diet of squid, anchovy or mackerel. CO_2 production and metabolism were measured with the oxygen-18 doubly-labeled water technique. Assimilated prey intake is the energy available in the ingested prey for a diet of anchovy and squid, followed by a diet of mackerel.

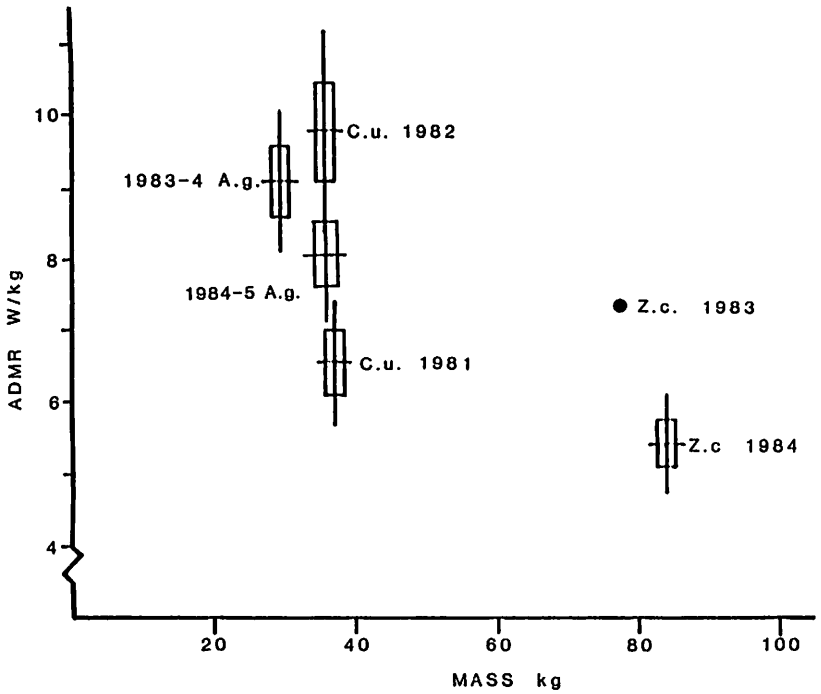

Figure 1. Average daily metabolic rate (ADMR) in watts per kilogram (1 W/kg = 0.0484 kcal/kg-day) for two fur seals (A.g. = Antarctic fur seal, *Arctocephalus gazella*; C.u. = northern fur seal, *Callorhinus ursinus*) and the California sea lion (Z.c. = *Zalophus californianus*) increases in response to reduced food availability in poor seasons (1982 for C.u, 1983 for Z.c.; 1983-84 for A.g) as compared to normal seasons (C.u. 1981, Z.c. 1984). (Notice that the mass specific metabolism is less for the larger sea lion than for the smaller fur seals.)

Such observations of animal responses to natural fluctuations in prey availability have obvious implications for discussions of the potential impact of commerical fisheries on important prey resources of sea lions.

Studies on Northern Elephant Seals

In 1983 studies were initiated to examine the foraging behavior of northern elephant seal by use of depth recorders (Le Boeuf et al., 1986). During 1985 and 1986 we extended these studies by attaching time–depth recorders to ten additional female elephant seals (3 in 1985 and 7 in 1986). In addition, we have deployed and are expecting the return of a depth recorder attached to a male elephant seal during August 1986. To supplement the depth of dive information, we injected six of these animals with tritiated water to estimate food consumption rates from water influx. Collaborating on this project were Drs. B. J. Le Boeuf, S. D. Feldkamp, and A. Huntley of University of California, Santa Cruz. The time–depth recorder (TDR) is a mechanical device that records on film a record of the diving pattern for up to 22 days. We successfully attached and recovered all units deployed and found that the animals dove routinely to 400 to 650 m, with a maximum depth of 820 to 850 m (Figure 2). This extends our previous record of 630 m measured in 1983, which had been the deepest diving range recorded for any pinniped. Diving was continuous throughout the first 14 to 26 days after the animals left the beach (the TDR recording duration)(Figure 3). Animals dove at a rate of 2.2 to 3.2 dives per hour (653 to 884 total dives per individual). Seventy-two percent of all dives were in the 12 to 22 minute interval with the longest submersion lasting between 32 and 48 min (Figure 4, 5). Mean surface intervals between dives were invariably 3 min ± 30 sec, resulting in a total surface time of only 11% (Figure 4). Surface intervals did not

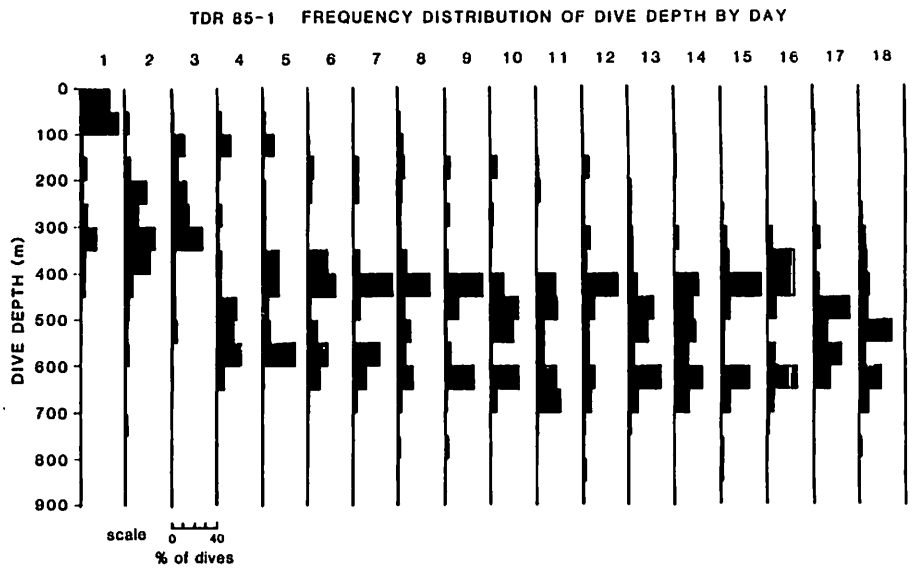

Figure 2. All female elephant seals were deep divers, with a bimodal distribution of diving depths per day between 400 and 650 meters. Maximum diving depths reached 850 meters. After 6 days at sea, dives less than 100 meters were very rare (0.06%) and only 1.3% of the dives were shallower than 200 meters.

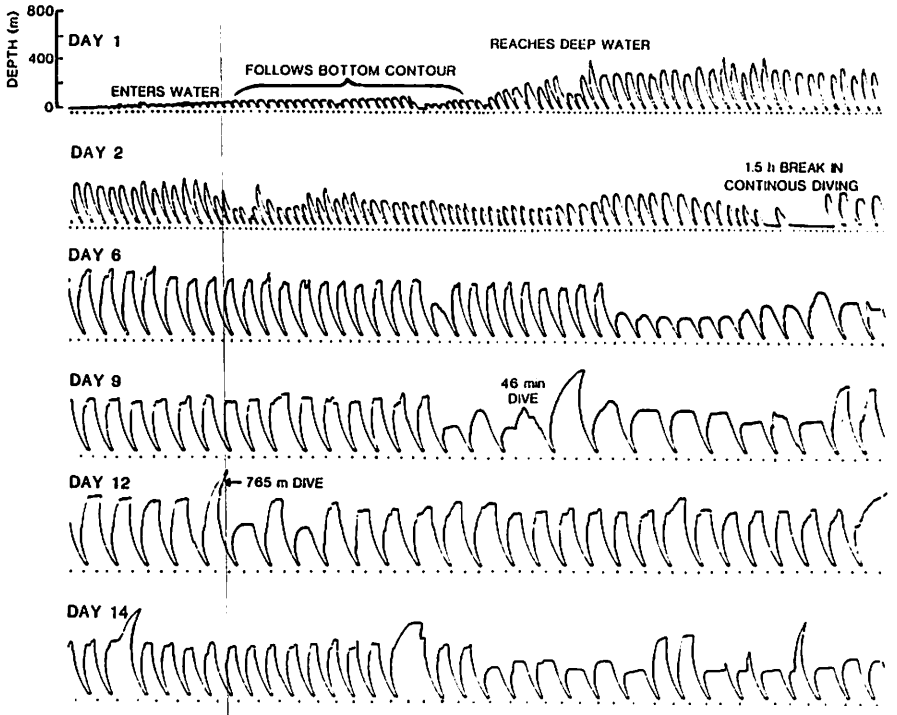

Figure 3. Representative excerpts of a time–depth record illustrate the fascinating continous diving pattern exhibited by all females studied. All subjects dove continuously 2.5 to 3 times per hour, each dive lasting approximately 20 minutes followed by 3 ± 1 (s.d.) minutes on the surface.

vary with length of the preceding dive. A pronounced diurnal diving pattern was observed with the deepest dives occurring during daylight and the shallower dives at night. There was no more than one rest period of 1 to 2 hr duration in any of the 12- to 20-day records. It appears that, at least during the initial period at sea, elephant seal females spend 89% of their time underwater!

Estimates of food intake over the entire trip to sea were determined from water influx data. Food intake was estimated by measuring the animals' field water influx using the tritiated water method (Nagy, 1975; Nagy and Costa, 1980). In this method food consumption is determined from the rate of water influx (Shoemaker et al., 1976). Water turnover measurements are made using tritiated water as previously described and validated for sea lions. The only difference is that a larger dose of tritiated water is required. In calculating food intake we assume that the seals do not drink, so that there are only two forms of water influx, metabolic water production and preformed water derived from the ingested food. Using the Shoemaker et al. (1976) equation that relates food intake to water turnover and assuming that the seals ate squid composed of 74% water, 5.11 kcal/g dry weight, and that elephant seals, like sea lions, metabolize 78% of the ingested squid energy, the mean daily at-sea prey consumption of these female seals ranged between 19.6 and 24.3 kg or between 4 and 7% of their body mass per day in prey (Table 6).

Our results have been used by Dr. Michael Bonnell who recently completed a 5-year marine mammal survey for the Minerals Management Service, Department of Interior. He was interested in the amount of time elephant seals spend underwater. The small number of elephant seals seen at sea after the breeding season did not compare with the large number of animals counted on the beaches during the breeding season. This led to speculation that elephant seals leave California waters immediately after the breeding season. However, this would have required an instantaneous departure. Our observation that elephant seals spend 89% of their time submerged reconciles this apparent discrepancy and indicates that elephant seals are more abundant than these aerial surveys indicate. Such surveys observe only one of every ten animals at the surface. These data have a profound influence on management questions relating to the seasonal abundance and movement patterns of this species in California.

Fisheries Implications

Although this work centered on only one period of the life history in

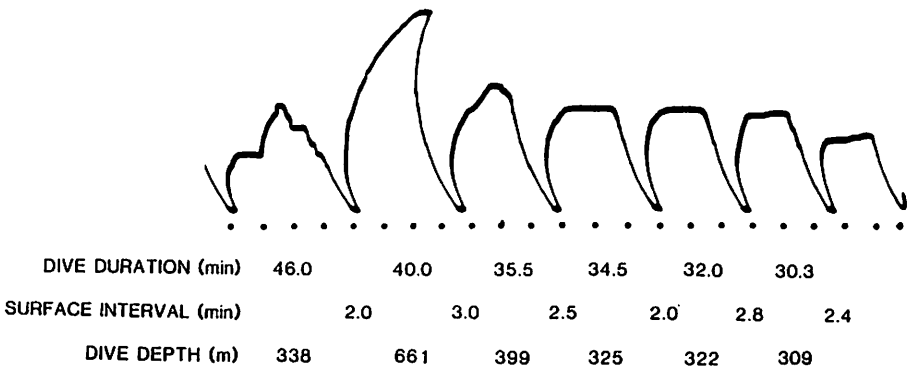

Figure 4. Female elephant seals spent between 83 and 88% of their time at sea underwater, or conversely they spent only 2.5 to 4 hours per day on the surface. Surface intervals were not correlated with dive duration even after 45 and 40 minute dives.

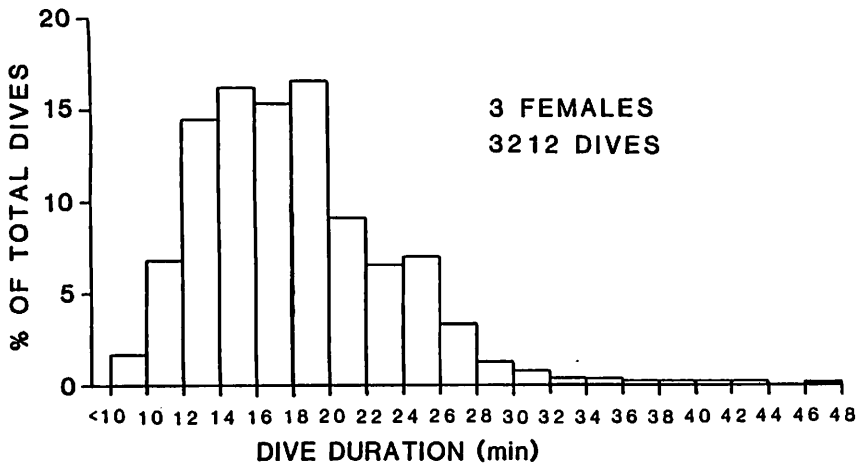

Figure 5. The duration of 72% of all dives was within 12 to 22 minutes, with only 6% of the dives being equal to or greater than 26 minutes.

these two species, some extrapolations can be made concerning the impact of these species on fisheries resources. From Table 5 we can see that the average sea lion female consumes 10.4% of her body mass daily in prey. Since the average female weighed 83.9 kg, she would consume 8.73 kg of fish per day foraging at sea. These data are only applicable for lactating females. Perez and Mooney (1986) have recently shown that while foraging at sea, lactating northern fur seal females consume 1.6 times as much food as non-lactating individuals. If we assume that such a relationship holds true for foraging sea lions we find that they would consume 6.5% of their body weight per day foraging at sea. The average body mass of territorial male California sea lions was measured to be 395 kg (Odell, 1981); therefore while at sea, male sea lions would consume 25.7 kg/day. Given the data on the distribution of California sea lions on the major Southern California breeding sites given by the work of Bonnell et al. (1978), we can estimate the daily at-sea food consumption of this species (Table 7). However, it is important to note that this is an estimate for the rate of food intake while these animals are actively foraging at sea. These animals spend considerable periods of time fasting onshore and thus the overall time spent foraging is considerably less. For example, female sea lions spend approximately 3 days with their pups onshore and 3 days at sea foraging. Therefore, averaged over the breeding season the daily food consumption would be half of this value. This relationship is even more confusing for males, since they may spend several weeks to over a month fasting on the rookery. The energy for this fast was stored from the preceding spring and was likely obtained from prey far outside of the coastal waters of Southern California.

Similar calculations can be made for northern elephant seals. Although detailed data divided by age and sex are not available, a minimum estimate of the number of females can be derived from pup counts. If we use the data in Table 6 on daily at-sea food intake of female elephant seals and multiply it by the minimum number of breeding females, we derive an estimate of the minimum food requirements of northern elephant seal females during the 74-day period spent at sea (Table 8). The data in Tables 7 and 8 show significant quantities of prey consumption by both northern elephant seals and California sea lions. However, the data on dive

Table 5. Results of Field Measurements Carried Out on San Miguel Island July 1984*

	Mass kg	CO_2 Production ml/g-hr	Metabolism kcal/kg-day	Water Influx ml/kg-day	Prey Intake % Body Mass per day	Assimilated Prey Energy kcal/kg-day
Female #184 7/12–17/84	79.5	0.911	132	93.3	10.6	123-155
Female #284 7/12–20/84	66.5	0.928	135	87.8	10.0	115-146
Female #384 7/15/84	92	–	–	–	–	–
Female #484 7/16–19/84	82.3	0.705	103	57.6	6.5	76-96
Female #584 7/20–27/84	91.5	0.578	84.4	99.9	11.3	131-166
Female #684 7/22–31/84	88.5	0.801	117	99.5	11.3	131-166
Female #784 7/22–28/84	77.3	0.723	105	96.9	11.0	127-161
Female #884 7/24–29/84	93.8	0.721	105	108.9	12.4	143-181
Mean	83.9	0.767	112	92.0	10.4	121-153
s.d.	9.4	0.123	17.8	16.5	1.9	22-27

*Prey intake as estimated from water influx is given as percent of the sea lions body mass per day on a diet of squid, anchovy or mackerel. CO_2 production and metabolism were measured with the oxygen-18 doubly-labeled water technique. Assimilated prey intake is the energy available in the ingested prey for a diet of anchovy and squid, followed by a diet of mackerel.

Table 6. Mass at Departure and Recapture, Time Spent at Sea Foraging, Water Influx Rates and Estimated Daily Food and Energy Intake (on a Squid Diet) of Six Elephant Seal Females.

Elephant Seal #	Mass Initial kg	Mass Final kg	Time days	Daily Gain kg/day	Water Influx ml/kg-day	Food Intake kg/day
1	300	380	71	1.13	61.7	24.28
2	348	424	80	0.95	62.7	24.20
3	426	480	73	0.74	43.3	19.61
4	237	305	76	0.89	66.9	20.98
5	325	384	63	0.94	51.0	20.74
6	253	318	78	0.83	59.7	19.70
Mean =	315	382	74	0.91	57.6	21.58

patterns indicate that elephant seals are obtaining their prey from depths far below that commonly used for commercial fishing.

In contrast during the summer in Southern California, sea lions feed primarily on market squid (70% by mass), Pacific whiting (15% by mass), juvenile rockfish (12% by mass), and northern anchovy (approximately 3% by mass) (Antonnelis, Fiscus and De Long, 1984; percentage data estimated from their Figure 4). Overall food consumption of breeding California sea lion females during July and August would be 6,780,000 kg of prey. This would likely be composed of 4,750,000 kg market squid, 1,020,000 kg Pacific whiting, 813,600 kg rockfish, and 203,000 kg northern anchovy.

Table 7. Maximum Number of Male and Female California Sea Lions on the Major Breeding Islands of Southern California (Bonnell et al., 1978)*

	Maximum Number Of		Food Intake While at Sea	
	Males	Females	Females kg/day	Males kg/day
San Miguel Isld	1199	13228	116000	30800
San Nicolas Isld	613	10921	95300	15800
Santa Barbara Isld	60	734	6400	1500
San Clemente Isld	72	995	8690	1850
Total	1944	25878	226000	50000

*Food consumption is estimated for females' from the data in Table 5, assuming that the animals studied in 1984 on San Miguel Island are typical of all sea lions. The food intake of males is estimated from a mean body mass of 395 kg (Odell, 1981) and a daily food intake of 25.7 kg/day while at sea. This is based on the females' food consumption corrected for the increased rate of energy required for lactation (Perez and Mooney, 1986).

Cooperating Organizations
Año Nuevo State Reserve, San Mateo County, California
Channel Islands Marine Sanctuary Program, Ventura, California
Channel Islands National Park, Ventura California
National Marine Mammal Laboratory, National Marine Fisheries Service, Seattle, Washington
Physiological Research Laboratory, Scripps Institution of Oceanography, La Jolla, California
Southwest Fisheries Center, National Marine Fisheries Service, La Jolla, California
Vancouver Public Aquarium, Vancouver, British Columbia, Canada

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Table 8. Population Estimates of Northern Elephant Seals Censused in 1978*

	Annual Pup Production	Total Animal Number	Adult Female Food Consumption kg/day	Adult Female Food Consumption kg/74 day
San Miguel Isl.	5455	24202	118000	8720000
San Nicolas Isl.	961	3598	20758	1536000
Santa Barbara Isl.	82	306	1800	131100
Farallones	133	580	2900	213000
Año Nuevo	1006	4588	21700	1608000
Total	7637	33274	165000	12210000

*Data on daily food intake of females was extrapolated from the number of females (assumed to be equivalent to pup production) and the calculated rate of mass consumed (from Table 2). The food consumed during the post-lactation 74 days at sea is also given. Food consumption is not estimated for the entire population, since we do not have data on population number and size of the different sexes and ages of the animals in the population.

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Enzymatic Degradation of Material from Shellfish Processing

San Diego State University
R/F-93
Project Initiated: October 1, 1984
Project Completed: September 30, 1986

Judith W. Zyskind

Chitin, an insoluble polysaccharide widely distributed in nature, is responsible for the rigidity and shape of the fungal cell wall and the cuticle of crustaceans and insects. It is composed of a linear array of beta-(1,4)-linked *N*-acetylglucosamine (GlcNac) units. Bacterial hydrolysis of chitin to GlcNac requires two enzymes: chitinase and *N,N'*-diacetylchitobiase (chitobiase). The overall project objective was to provide methods for producing these enzymes in large amounts using genetic engineering techniques. In the last three years, we have cloned the genes encoding these enzymes from the marine bacterium *Vibrio harveyi* and the terrestrial bacterium *Serratia marcescens*, using plasmid and cosmid vectors. We have found that each organism contains one chitobiase gene and two chitinase genes, all unlinked to each other. Another research group (Jones et al., 1986) has also cloned two different chitinase genes from *S. marcescens* and has determined the nucleotide sequence for one of them. Because this is the completion report, significant results obtained throughout the granting period are included; however, the results of the last year are presented in greater detail.

Synthesis of Chitinase and Chitobiase in *V. Harveyi*

The pattern of chitinase and chitobiase synthesis by *V. harveyi* when grown in the presence of chitin was followed in order to determine the optimum time for isolating these enzymes. *V. harveyi* was grown in minimal medium containing 15 mg/ml swollen chitin for five days. Samples were removed every 12 hours and examined microscopically for the disappearance of chitin and evidence of cell lysis. Cell viability was assayed by colony plate counts of sample dilutions. Cells were concentrated, disrupted by sonication, and the supernatants and cell extracts were assayed for chitinase and chitobiase activities. Results from this experiment indicate that (1) extracellular chitinase activity is the result of secretion and not cell lysis; (2) chitinase activity peaks at 48 hrs and chitobiase activity peaks at 36 hrs; and (3) chitobiase activity is found associated with cells at all time points.

We have subsequently demonstrated that chitobiase activity is associated with the membrane fraction of sonicated cell preparations of both *V. harveyi* cells and *E. coli* cells containing the *V. harveyi* chitobiase gene on a plasmid.

Chitobiase: Size, Gene Cloning, Sequence Analysis

Part of the recent focus has been to determine physical and biochemical properties of the chitobiase gene (*Chb*) and enzyme (Jannatipour, et al., 1987). The molecular weights of the unprocessed and processed forms of chitobiase are 95.3 kDa (kilodaltons) and 92.1 kDa, respectively, based on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins labeled with ^{35}S and isolated from minicells containing pRSG14 and pRSG16 (Jannatipour, et al., 1987). Assuming the average size of an amino acid to be 110 daltons, the size of the chitobiase gene should be approximately 2.4 kb (kilobase pairs) in length. We have used the plasmid pUC19 (Yanisch-Perron Vieria, and Messing, 1985) for subcloning the chitobiase gene. This plasmid is part of a series of plasmid vectors containing the alpha fragment of the *E. coli* gene for beta-galactosidase, which complements a deletion of the amino-terminus of the *lacZ* gene. Restriction fragments inserted into the polylinker downstream of the *lac* promoter interrupt the reading frame of the alpha portion of the *lacZ* gene and give white instead of blue colonies on indicator plates. A 3.5 kb *Eco*RI restriction fragment of pRSG14 was inserted in both orientations into the *Eco*RI site in the polylinker of pUC19. These plasmids (pRSG192 and pRSG191) show constitutive expression of chitobiase with or without the addition of chitobiase. If the inducer (IPTG) of the *lac* promoter is added, however, increased expression is noted in only one orientation (pRSG192), indicating the direction of transription of the *Chb* gene. If a small 0.5 kb *Sst*I fragment that includes the *Eco*RI site at one end of the *Eco*RI fragment in pRSG192 is deleted (plasmid pRSG196), chitobiase activity drops approximately 12-fold. The addition of IPTG partially restores chitobiase activity in pRSG196, indicating that the transcription direction of the chitobiase gene in pRSG192 and pRSG196 is the same as for the *lac* promoter and that a sequence with promoter activity has been deleted from pRSG196. Minicell analysis of ^{35}S-labeled polypeptides encoded by pRSG192 shows two species of polypeptides, 93.5 and 92.1 kDa, whereas only a 93.5-kDa polypeptide was labeled in minicells containing pRSG196. The sequence of most of the 3.5 kb *Eco*RI fragment containing the chitobiase gene has been determined, and an open reading frame corresponding to a polypeptide 96-kDa in size appears in this sequence. Based on DNA sequence and open reading frame

analyses, the probable amino-terminus of the unprocessed enzyme is composed of the following amino acid sequence:

MetLeuLysHisSerLeuIleAlaAlaSerVal
IleThrThr*LeuAlaGlyCysSerSer*Leu
GlnSerSerGluGln

The italicized amino acids are identical to the amino acids adjacent to the processing site in the outer membrane lipoprotein of *E. coli* (Braun and Bosch, 1972; Nakamura and Inouye, 1979) and may represent the region where processing of chitobiase occurs.

Chitobiase is Located in the Outer Membrane of *E. coli* Cells

Fractionation studies of *E. coli* cells containing pRSG192, where cells were separated into membrane, cytoplasmic, and periplasmic fractions, located the chitobiase activity in the membrane fraction (Jannatipour et al., 1987). The membranes were further separated using an Osborn gradient (Osborn et al., 1972) into inner and outer membrane fractions and assayed for chitobiase and the inner membrane enzyme, D-lactate dehydrogenase. The major portion of the chitobiase activity was recovered in the outer membrane fraction, and this observation led to a simple two-step purification scheme described below. When similar studies were performed with *E. coli* cells containing pRSG196, 98% of the chitobiase activity was located in the cytoplasm. From the nucleotide sequence of the chitobiase gene and the cloning vector, pUC19, it was possible to determine that the *Sst*I deletion in pRSG196 created an in-frame fusion of the amino-terminus of the *lacZ*, replacing the first 22 amino acids of chitobiase. The minicell results and cell fractionation studies with the two plasmids, pRSG192 and pRSG196, establish that chitobiase is processed by removal of a signal peptide during transport to the membrane and that processing occurs at the amino-terminus with sequences at the amino-terminus determining the specificity of processing.

Purification of Chitobiase

When we found that chitobiase was located in the outer membrane, we examined the application of known methods for purifying other outer membrane proteins, such as treatment of whole cells with detergents (Soto-Gil et al., in press). It was found that *N*-lauroylsarcosine (sarcosyl) removes approximately 75% of the chitobiase activity from cells at a concentration of 1%. This concentration had no effect on the activity of chitobiase, so it was used in the first step to remove chitobiase from *E. coli* cells containing the plasmid pRSG192. The cells are centrifuged, washed twice with 50 ml of 10 mM Tris-Cl (pH 7.3), and resuspended in 100 ml of 10 mM Tris-Cl (pH 7.3) containing 1% w/v sarcosyl. After incubating for 1 hr at 37°C with gentle mixing, the cells are removed by centrifugation at 27,000 x g for 15 min. The supernatant fluid, called the sarcosyl cell extract, is stored at –20°C; there is no loss of chitobiase activity over a two-week period under these conditions. This first step isolates chitobiase free from most of the *E. coli* proteins because it does not involve cell lysis; the contaminating proteins, which are mainly other outer membrane proteins, are removed in the next step.

The second step involves HPLC purification of chitobiase. Preparative isolation of chitobiase was carried out on a 4.6 x 250 mm column of Waters QMA ACCELL ion-exchange medium (37–55 µm, 500 nm pore size). The packed column was washed successively with methanol (50 ml), acetonitrile, 0.1% trifluoroacetic acid in nano water, and nano water before equilibration with 200 ml of 20 mM sodium acetate (pH 6.5, solvent A). An absorbance baseline for the column was established by running a linear gradient from 0.0 to 0.1 M NaCl in 20 mM sodium acetate (pH 6.5, solvent B) over 45 min at a flow rate of 2 ml/min.

Aliquots of the sarcosyl-cell extract (2 x 5 ml, 0.4 mg/ml) were loaded onto the column at 1 ml/min and washed through with solvent A until a baseline 260 nm absorbance (0.5 AUFS) was obtained. The flow rate was increased to 2 ml/min before initiation of the following gradient program: 0–5 min, 100% A; 5–12 min, 0–25% B; 12–40 min, 25 to 65% B; 40–45 min, 65–100% B; 45–50 min, 100% B; 50–55 min, 100% B to 0% B. Three ml fractions were collected. The enzyme eluted in the 8–14 min region of the gradient program. The purified enzyme has a specific activity of 107 units/mg and can be obtained in up to 30% yield. Coomassie Blue-stained SDS-polyacrylamide gels exhibited a polypeptide with a Mr = 90,000 comprising about 95% of the applied protein. This method is summarized in a recent publication (Soto-Gil et al., 1988).

Studies with chitobiase purified using the protocol described above demonstrated that the enzyme cleaves both chitobiose and the artificial substrate, p-nitrophenyl-2-acetoamido-2-deoxy-B-D-glucopyranoside (PNAG), but with differing apparent Vmax's; the native substrate is hydrolyzed with a turnover number 7.5-fold greater than that for the artificial substrate. The pH optimum at 25°C in Tris-Cl is broad over the pH 6-9 region. The enzyme exhibits classical Michaelis Menten kinetics and an apparent Km at 25°C for the artificial substrate of 250 µM. The enzyme is unaffected by a variety of cations including Na^+, NH_4^+, K^+, and Mg_2^+ and can be assayed in citrate or phosphate buffers. The enzyme is unaffected by the presence of 10 mM EDTA and exhibits 80% of its original activity in 1 M NaCl. The enzyme can be stored at 4° or at –20° for up to two weeks without loss of activity, and lyophilization results in 40 to 50% loss of activity.

Insertional Mutagenesis of *V. harveyi*

Three types of *V. harveyi* mutants that would be of significant value to this study are constitutive chitinase mutants, chitinase secretion mutants, and chitinase-negative mutants. To isolate such mutants, *V. harveyi* was transduced via P1

with Mini-Mu *tet lac* (Belas et al., 1984), a Mini-Mu derivative obtained from M. Silverman. Fifty thousand tetracycline-resistant colonies were replicated to chitin-containing medium, and, after three days of growth, the colonies were washed from the surface of the agar. Only two phenotypes were recovered, chitinase overproducers (22 isolates) and very low chitinase producers (33 isolates). No chitinase-negative mutants were recovered; this is probably because there is more than one gene encoding chitinase activity in *V. harveyi* (see below).

The mutants that produce very little chitinase have been further characterized with regard to extracellular enzyme production. The culture supernatant fluids of 24 of these mutants grown for 48 hrs were precipitated with 20% acetone. The precipitated proteins were separated by SDS-polyacrylamide gel electrophoresis to determine their sizes and relative amounts. The largest class (16/24) contained extracellular proteins in the same relative amount and distribution as wild-type *V. harveyi* except that they do not synthesize chitinase above the noninduced levels. These mutants are probably defective in the regulation of chitinase expression, and they will be used in subsequent studies to determine the regulatory mechanism(s) involved in the control of chitinase gene expression. The other two classes of mutants either did not contain any extracellular proteins (4/24) or were missing one or more of the extracellular proteins (4/24). These mutants are probably deficient in the secretion of extracellular proteins and will be important in establishing how chitinase is secreted across the Gram-negative double membrane of *V. harveyi.*

The chitinase overproducers all synthesize chitinase constitutively, but chitobiase activity is inducible, indicating independent control of chitinase and chitobiase gene expression. One of the chitinase overproducers, which synthesizes one of the chitinases of *V. harveyi* constitutively, was used in the purification of chitinase activity as described below.

Purification of a Chitinase Activity from *V. harveyi*

For purification, 500 ml cultures of the *V. harveyi* chitinase over-producing mutant BB7-1 were harvested after 3 days of growth at 30°C. The procedure used is similar to one described by Roberts and Cabib (1982). Cells were removed from the culture by centrifugation and the filtrate was concentrated 10-fold in an Amicon Pressure Cell with a YM30 filter. Swollen chitin was added to the enzyme at a concentration of 0.15 mg chitin per unit of chitinase and the mixture incubated 1 hr on ice. After centrifugation at 25,000 g for 30 min, the pellet was washed with twice the original volume of 10 mM Tris-Cl, pH 7.3 prior to suspension in the original volume of the same buffer plus 10 mM sodium azide and 50 g/ml chloramphenicol. After incubation for 12 hrs at 30°C with shaking, the remaining particulate material was removed by centrifugation and the supernatant fluid was dialyzed to remove the reaction products.

This one-step affinity procedure employed for purification of one of the *V. harveyi* chitinases is based on the binding of the soluble enzyme to the insoluble substrate, chitin. In one such purification, about 53% of the chitinase activity was recovered with a 162-fold purification. When the purified chitinase obtained by chitin absorption was examined by SDS-polyacrylamide gel electrophoresis, there was only one major band corresponding to a molecule weight of 85,000.

Properties of One of the *V. harveyi* Chitinases

To probe the thermostability of the chitinase purified as described above, chitinase was exposed to three different temperatures for varying periods of time and then assayed for the remaining residual activity at 30°C. The results demonstrate that this chitinase is indeed an extremely heat stable enzyme with a half-life of 40 min at 95°C. It had been suggested to us that chitinase might be insensitive to detergents because some of the extracellular enzymes of *S. marcescens* do not lose activity when exposed to SDS (S. Molin, personal communication). The purified chitinase from the *V. harveyi* overproducing mutant, BB7-1, was exposed to different concentrations of SDS ranging from 0 to 0.1%; the concentration in SDS-polyacrylamide gels is 0.1%. After 1 hr exposure to SDS on ice, chitinase activity was determined in the presence of the same concentration of SDS. One hundred percent of the activity was present after treatment with all concentrations of SDS, clearly indicating that chitinase is far more resistant to denaturation by SDS than other enzymes. Treatment of chitinase with SDS at higher temperatures, however, resulted in the accumulation of smaller molecular weight material. This was probably due to unfolding of the enzyme by SDS, which caused a new sensitivity of chitinase to contaminating proteases.

Cloning of *V. harveyi* and *S. marcescens* Chitinase Genes

Because there were no chitinase-negative mutants recovered in the collection of 50,000 Mini-Mu insertions of *V. harveyi* and because other researchers had found two chitinase genes in *S. marcescens* (Jones et al., 1986), we looked for chitinase genes in *V. harveyi* other than the one that had been cloned in the first genomic band (pRSG125) on an 8.8 kb insert. A cosmid vector, pMMB33 (Frey et al., 1983), was used to construct genomic libraries of *V. harveyi* strain B392 and *S. marcescens* strain QMB1466 (Monreal and Reese, 1969) containing DNA fragments 30 to 40 kb in size. In the 1200-member *V. harveyi* library, there were 27 chitinase-positive clones and 7 chitobiase-positive clones, and in the 1000-member *S. marcescens* library, there were 20 chitinase-positive clones and 2 chitobiase-

positive clones. None of the chitinase-positive clones contained chitobiase activity, and none of the chitobiase clones contained chitinase activity. Because of the size of the inserts in these genomic libraries, this result clearly demonstrates that the genes encoding these two activities are unlinked. Two different and non-overlapping restriction digest patterns were obtained with a subset of the *V. harveyi* chitinase-positive clones, one identical to pRSG125 and one with a new restriction-digest pattern. The sizes of these two different chitinase genes are being determined by subcloning and nucleotide sequence analysis. In the *S. marcescens* chitinase-positive clones, at least 2 separate categories of restriction digest patterns could be discerned and two of these patterns resemble the published patterns for the two *S. marcescens* chitinase genes previously isolated (Jones et al., 1986). The specific activity of one of the *S. marcescens* chitinases appears to be higher than for the other chitinases. We plan to insert this chitinase gene into a plant-cloning vector in collaboration with Stephen Howell at UCSD to examine whether it induces fungal resistance and to determine where it becomes localized in the plant.

Summary

V. harveyi and *S. marcescens* each have one chitobiase gene and two chitinase genes, all unlinked. These genes have been cloned onto plasmids in this study.

A detailed analysis of the *V. harveyi* chitobiase gene and enzyme has been completed. The chitobiase gene was cloned with its promoter intact; however, it is expressed constitutively in *E. coli*, probably because it has been separated from genes that control the inducibility of the enzyme in *V. harveyi*. The gene has been sequenced, and the putative amino acid sequence of the enzyme was deduced from the nucleotide sequence. Chitobiase is located in the outer membrane of *E. coli* cells containing the *V. harveyi* gene, and a signal sequence is removed from the amino-terminus during transport.

The results with the four different chitinases are still preliminary, partly because of the difficulty in analyzing two enzymes with the same activity in the same cell. We have constructed tools that will be useful in future analyses of these enzymes including: (1) plasmids containing the different chitinase genes from *V. harveyi* and *S. marcescens*, (2) *V. harveyi* mutants that appear to constitutively express either one or the other chitinase enzymes, (3) *V. harveyi* mutants that do not produce increased chitinase in the presence of chitin, in either one or the other chitinase, and (4) *V. harveyi* mutants that appear to be deficient in the secretion of extracellular proteins, including chitinase.

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Freezing-Induced Changes in Fish Tissue

University of California, Davis
R/F-95
Project Initiated: October 1, 1983
Project Completed: September 30, 1985

Davis S. Reid

In 1984-85, several batches of rockfish, obtained directly from fishing vessels within hours of catching and transported immediately to Davis on ice, have been prepared for freezing, and have been frozen either rapidly or slowly. Individual fish have then been stored at one of three storage temperatures, –5°C, –12°C, or –20°C. Prior to freezing, material has been analysed to obtain a chemical/biochemical characterization of the fresh fish, and small samples have been fixed to allow for microscopic characterization of the fresh material. Samples of fish immediately post-freeze have been assayed, and the changes taking place during extended storage have been monitored.

In order to follow the changes in the structure of the frozen fish and to assess the structural damage consequent upon ice formation, a specialized fixation method has been used to render the fish tissue suitable for further optical or electron microscopic examination. This specialized fixation technique, known as isothermal freeze fixation (Asquith and Reid, 1980), was first applied to fish in an associated rapid response project and has been further developed in this study. The special fixative does not disturb the ice present in the tissue.

Different microscope techniques are appropriate to investigate different aspects of the frozen tissue. Optical microscopy allows us to visualize the ice crystals easily, and we should therefore be able to determine the amount, size, number, and location of the ice crystals. We are building up a set of slides for optical microscopy, which show the change in ice crystal character as a function of freezing method and storage time, and also clearly show the effect of storage temperature on the amount of ice in the tissue. Quantification of the micrographs is planned.

Scanning Electron Microscopy gives us a three dimensional view of the frozen tissue and clearly illustrates ice damage. Transmission Electron Microscopy allows us to visualize the change in the matrix between ice crystals consequent upon ice formation. The higher concentration of the matrix at lower temperatures is readily apparent.

Much data analysis is required before the information contained in the photomicrographs can be fully appreciated. We hope to base our final conclusions on objective quantitative analysis of the photomicrographs, rather than on qualitative comparisons between pictures, but this depends on the suitability of the micrographs for automated image analysis.

The microstructural data, in particular relating to tissue damage and complexity of the pathways between ice crystals, are to be examined also in the light of data generated on the chemical/biochemical deterioration of the fish in storage. We have not been entirely successful in miniaturizing our assays in order to perform a wide range of studies on one fish. Sampling variation has meant that most assays perform best on samples of 10 g upwards. We have, therefore, followed particular chemical/biochemical indicators on individual fish, rather than following all indicators on each fish. As expected, protein solubilities have decreased with extended storage, and the rate of loss of solubility was greater at higher temperatures. Gel electrophoresis of the proteins indicates changes in the aggregation patterns of the myosin proteins, in particular, during storage. The ATPase activity of the myosin is also lost during storage—again the loss is more rapid at the higher storage temperatures. The pH of the tissue does not change significantly. The tissue does, however, lose water increasingly as a function of frozen storage time. While volatile amine production has been observed, the amounts produced are uncertain, as we are close to the resolution limits of the GC method we are employing (Lundstrom and Radicst, 1983).

We have extracted the lipids from some fish samples, and the results are indicative of change. Identification of individual lipids, and lipid degradation products by GLC and by thin layer chromatography methods (Khayat and Schwall, 1983) is in progress. Crude tests indicate increasing oxidation, the extent being dependent on freezing conditions.

Studies to relate frozen structures with precise freezing conditions are being initiated. Cryomicroscopy allows us to observe freezing under controlled conditions, and we are attempting to determine the extent of artifact production consequent upon isothermal freeze fixation.

As storage continues, we are extending our data base on both biochemical change and structural change. The identification of correlations, should they exist, is dependent upon the establishing of clear trends. Data collection is still an important part of the project. We are now, also, well into a data analysis phase.

Cooperating Organizations

Campbell Soup Company
University of California, Davis
Experiment Station

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Genetic Improvement of a Chitinase-Producing Microorganism

University of California, Davis
R/F-96
Project Initiated: October 1, 1983
Project Completed: September 30, 1985

David M. Ogrydziak

A library of *S. marcescens* DNA was constructed in the bacteriophage lambda. Plaques were sprayed with the chitobiase substrate nitrophenyl-N-acetyl-glucosamine and several recombinant phage from each of two different regions of the genome were obtained (Horwitz and Ogrydziak, 1984). Chitobiase activity was demonstrated in phage lysates of phage from one region and not the other. Endochitinase activity could not be reproducibly demonstrated in any of the phage lysates. Attempts to find the endochitinase gene by screening the library on chitin-impregnated agar plates were unsuccessful.

Restriction maps were constructed of several of the inserts containing the chitobiase gene. Attempts to subclone an 11 kb fragment into pBR329 were unsuccessful. A 3.3 kb fragment containing the chitobiase gene was cloned into the *Eco*R1 site of pBR329 to produce plasmid pXBi. Initial attempts to transform this plasmid into *S. marcescens* QMB1466 (a strain which produces high levels of the chitinase enzyme complex) were unsuccessful. The plasmid was transformed into a restriction minus *S. marcescens* strain TT392 which was obtained from Japanese scientists. The strain had been heavily mutagenized and did not produce chitobiase or endochitinase. The plasmid pXBi prepared from TT392 gave about 100 times higher transformation frequency in *S. marcescens* QMB1466 than did pXBi prepared in *Escherichia coli* HB101. This strongly suggests that a restriction system in *S. marcescens* QMB1466 was reducing the transformation frequency. Plasmid miniscreens DNA of the QMB1466 transformant had the same *Eco*R1 digestion pattern as pXBi. Experiments were initiated to determine the level of chitobiase production by *S. marcescens* QMB1466 containing pXBi.

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Genetic Analysis of Anchovy and Sardine Populations in the California Current System

University of California, Davis
Bodega Marine Laboratory
R/F-98
Project Initiated: October 1, 1984
Project Completed: September 30, 1986

Dennis Hedgecock

For fishes such as many clupeoids that are both heavily fished and subject to vast natural fluctuations in recruitment and abundance, annual quotas on the harvest are set from yearly estimates of spawning biomass. This management strategy is flawed for any case in which it is not known whether the particular stock is homogeneous or a mixture of subpopulations. The collapse of the Pacific sardine fishery has been attributed to the sequential collapse of subpopulations (Radovich, 1982).

We proposed to study the population structure of two pelagic clupeoids in the California Current system, the northern anchovy (*Engraulis mordax*) and the Pacific sardine (*Sardinops sagax*). We undertook a major project with the anchovy because our parent project (UCSG R/F-82) had demonstrated that the heavily fished central stock was not homogeneous. This conclusion was based on genetic heterogeneity revealed by the analysis of 12 biochemical characters (enzyme phenotypes revealed by electrophoresis). To establish the sources of the heterogeneity, in the hope of obtaining a precise understanding of population subdivision, in the present study we assayed much larger samples (n = 120) for the same biochemical characters; to look for heterogeneity in other types of characters, we also took 16 morphological measurements on each fish. We assigned each fish to a relative age class from counts of annuli in otoliths and recorded the sex of each fish that was in reproductive condition. Seventeen samples collected in 1984 and 1985 were included.

Preliminary analyses of the northern anchovy morphometric data included regressing each character against standard length to reveal both experimental errors and differences in variance or other heterogeneity among samples. We found evidence of population heterogeneity both within samples and between nearby samples. Figure 1 gives an example. The samples were less than 50 km apart, from inshore southern California waters. Two size groups are seen. In the San Clemente sample, all but one or two of the O-annulus fish are in the group of smaller fish and all but one of the 1- and 2-annuli fish are among the larger fish; But in the Oceanside sample, all 0's, all but four 1's, and two 2's are among the smaller fish. The 1- and 2-annuli smaller fish in this sample may be of different provenance from those in the San Clemente sample. Distributions of standard lengths within sample annuli classes have coefficients of variation (standard deviation divided by mean) that are highly variable, and many distributions show bimodality or extreme skew. The between-sample variation in standard deviation is almost significantly greater than within-population among-annuli class variation (ANCOVA of standard deviation regressed upon mean standard length, $F = 1.80$, d.f. = 16, 42, $P = 0.06$); thus it appears that while some samples are homogeneous, others are not. This variation in heterogeneity adds another dimension of complexity to the analysis.

The northern anchovy biochemical data have been sorted into 130 subgroups, each constituted of fish of a single sex and a single annuli class from a single sample. For each subgroup, allelic frequencies have been calculated for 10 enzyme-coding genes. Analysis of cross-classified categorical data (Fienberg, 1980) will be used to compare these frequencies and also frequencies for such subgroups of the 1982 and 1983 data previously collected. Larger groups will then be formed for further analysis as appropriate.

The relationship between the morphometric and biochemical data from the northern anchovies is being examined with canonical correlation analysis, a technique that finds the maximum correlation between linear combinations of two sets of variables (Blackith and Reyment, 1971). Analyses prerequisite to applying this technique have shown that the distributions of the morphometric variables are close to normal, and that these data are highly collinear. A canonical correlation analysis of a subset of the data (n = 513) gave an r^2 of 0.12. We are exploring the possibility of performing canonical correlation analysis using—rather than the morphometric data themselves—loadings for each individual on sheared principal components (a multivariate statistical technique discussed below).

One hypothesis that we proposed to test had been formulated by Parrish (1983). He suggested that the observed heterogeneity in the central stock might be owing to its being subdivided into autumn- and winter-spawning subpopulations that are temporally, although not spatially, reproductively isolated from each other. Fewer than one percent of 2,420 anchovies that were caught during the months of January through March (1983–1985) were not in active reproductive condition. If our samples had been from a mixture of temporally reproductively isolated stocks, the fall-spawning fish would have been represented by individuals lacking

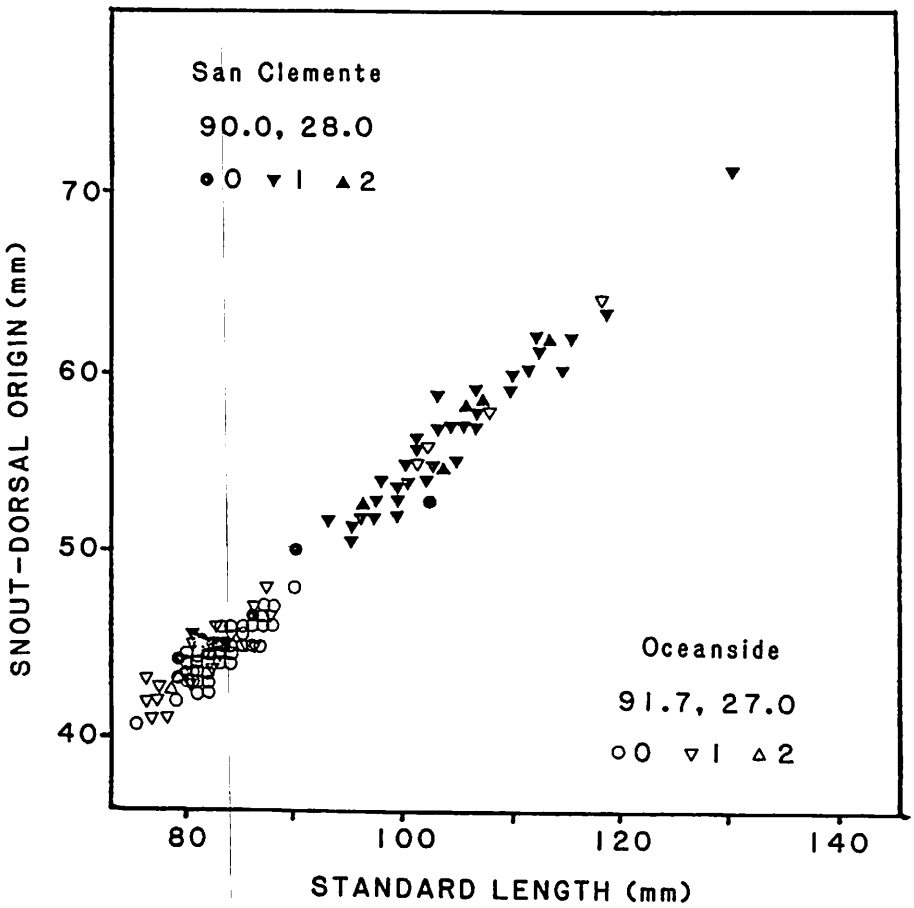

Figure 1. Regression of distance from tip of snout to dorsal fin origin on standard length for two inshore samples of northern anchovy collected less than 50 km apart (nearest shore localities and CalCOFI coordinates are given). 0, 1, and 2 are classes based upon the number of surficial otolith annuli.

gonads. To address the hypothesis, we had proposed to compare morphometric and biochemical data on samples collected in the autumn by commercial boats with data on samples from the winter cruises of the National Marine Fisheries Service. For economic reasons, the anchovy fishery ceased to function at about the same time our project began. We were able to obtain only a single autumn sample; the collection date of this sample, 14 November 1985, was later than we had hoped for. Preliminary analyses give no evidence that variation in this sample differs from variation in the winter samples. Distributions of all of the morphometric variables from this sample are similar to the corresponding distributions from at least two winter samples, which were collected in March. Locus by locus, the allelic frequencies in this sample, taken as a single group, are similar to those of winter samples; levels of heterozygosity are also similar. Assuming that the fish we studied are a valid random sample of the central stock, we can reject the hypothesis of subdivision into autumn- and winter-spawning races.

We conducted a much smaller study of the Pacific sardine; the goal was to ascertain whether biochemical or morphometric characters carry evidence that might be used to reconstruct the racial background of the sardine whose numbers are now increasing off California (Wolf and Smith, 1985).

Our assays of widely separated samples of the Pacific sardine reveal that there is so little within- and between-sample genetic variation for enzyme phenotypes that these biochemical characters would not be useful for reconstructing the racial backgound of populations. Table 1 gives the levels of polymorphism and heterozygosity we found for our samples; the few heterozygous fish do not reveal notable patterns of differences among the samples. This species is somewhat exceptional in having such low levels of polymorphism and heterozygosity. Part C of Table 1 compares these levels with those of northern anchovy, which are more typical of marine animals.

Species that are virtually devoid of between-population genetic variation for such biochemical characters are not necessarily without between-population environmentally induced or genetic variation for morphometric characters. We have learned that in this species morphometric characters are at least more promising than biochemical characters for separating subpopulations.

The collection points of the four sardine samples we studied morphometrically are well separated geographically: Tomales Bay, California (n = 5); Monterey Bay, California (n = 29); Magdalena Bay, B.C.S., Mexico (n = 37); and Guaymas, Sonora, Mexico (n = 48). There is a strong relation between the average standard length of sardine populations and latitude, with longer fish being found at higher latitudes (Radovich, 1982). As the geographical separation makes appropriate the *a priori* hypothesis that fish in the four samples are different, discriminant-function analysis of our multivariate data could be performed. Using 13 untransformed morphometric variables, we found that each of the four samples differs significantly from each of the other three. Further analyses in which we attempted to remove the effects of size produced the conclusion that only size differences and no shape variation could be detected among contemporary Pacific sardine populations (Hedgecock et al., 1985). We faulted this conclusion, however, upon the realization that we may have introduced correlations with standard length into our

transformed variables as described in the argument against the use of ratios in morphometrics (Humphries et al., 1981).

With the software available on the small computers at our remote facility, we could not perform state-of-the-art morphometric analyses (Bookstein et al., 1985). The ranges of standard length in our data for the sardines from Mexico and from California are virtually nonoverlapping. The two Mexican samples, however, do overlap in size; as Magdalena Bay is on the west coast of Baja California and Guaymas on the Gulf of California, the *a priori* hypothesis that the two samples differ is appropriate. We therefore performed discriminant function analyses on only fish of the same size from these two samples; the range of standard length for these fish spanned less than 2 mm (145-162 mm). The technique sorted the fish very well, with only 3 of 46 fish being misclassified (Figure 2). Examination of the way the variables loaded on the functions suggested that bony structure differences affecting the shape of the jaw were involved in the sorting. We show, in Figure 2, the pattern for the two variables with the highest loadings, maxillary length and interorbital width, by plotting their ratio on the ordinate.

With large samples and appropriate statistical software, other size-free shape differences might be found among populations of the Pacific sardine. If all or most of the samples for such a study were caught off the coast of California, assuming *a priori* that the samples were different would not be suitable. In this case, as for the analysis of our anchovy morphometric data, the multivariate method of choice would be sheared principal components (Humphries et al., 1981; Bookstein et al., 1985). We have been advised that software for running sheared principal components analyses on small computers will soon be available (J. Humphries, personal communication).

On the basis of morphometric, growth, and serological evidence, several authors have concluded that, prior to its collapse in abundance, the Pacific sardine comprised two or more distinct subpopulations. Population structure likely played a role in the collapse of the fishery, perhaps directly by virtue of differences in life histories and resilience to fishing pressure among subpopulations, or at least indirectly as unrecognized immigration of southern subpopulations caused an overestimation of stock size in the waning years of the fishery. With the return of substantial numbers of Pacific sardines in recent years has come interest in questions of *which* sardine population has recovered and what life history characteristics and yields can be expected.

Variation in size at age may be of more consequence for fisheries than anything else. The mean standard lengths in mm (and standard deviations) of fish in our samples are

Tomales Bay	237	(6.12)
Monterey Bay	202	(18.10)
So. Calif. Bight	211	(11.64)
Magdalena Bay	165	(8.96)
Guaymas	150	(6.07)

From these average sizes and the similarity in age composition of our samples as determined from annuli in otoliths, it would appear that geographical variation in growth rate today is at least as large as that

Table 1. Enzyme-Phenotype Variation in Five Population Samples of Pacific Sardines and Comparisons of Levels of Variability with Northern Anchovies. (Where two or more sardine samples are polymorphic, they share the same rare alleles, except for a unique 6-PGDH allele in the Guaymas sample.)

A. Summary Statistics	Sample				
	Tomales Bay	Monterey Bay	Magdalena Bay	Guaymas	So. Cal. Bight
No. of fish	5	29	37	48	30
No. of loci	27	27	27	26	26
% loci polymorphic/pop	7.4	14.8	7.4	26.9	15.4
% loci heterozygous/fish	1.5	1.0	0.5	1.7	1.3

B. Polymorphic Enzymes	No. of heterozygous fish in samples				
	Tomales Bay	Monterey Bay	Magdalena Bay	Guaymas	So. Cal. Bight
EST	0	–	–	3	–
FBP	1	–	–	7	0
-GPDH	0	1	0	0	0
IDH	0	0	0	1	1
PEP-LGG	0	–	0	1	0
PEP-LV/LT	0	–	0	1	0
PEP-PP	0	3	1	4	0
6-PGDH	1	3	4	4	3
TO	0	1	0	0	0
ME	0	0	0	0	6

C. Comparisons with Anchovy	Sardine	Anchovy*
No. of loci	33	41
% loci polymorphic/pop	13.7	36.6
% loci heterozygous/fish	1.2	7.9

*Source: Hedgecock and Li 1983

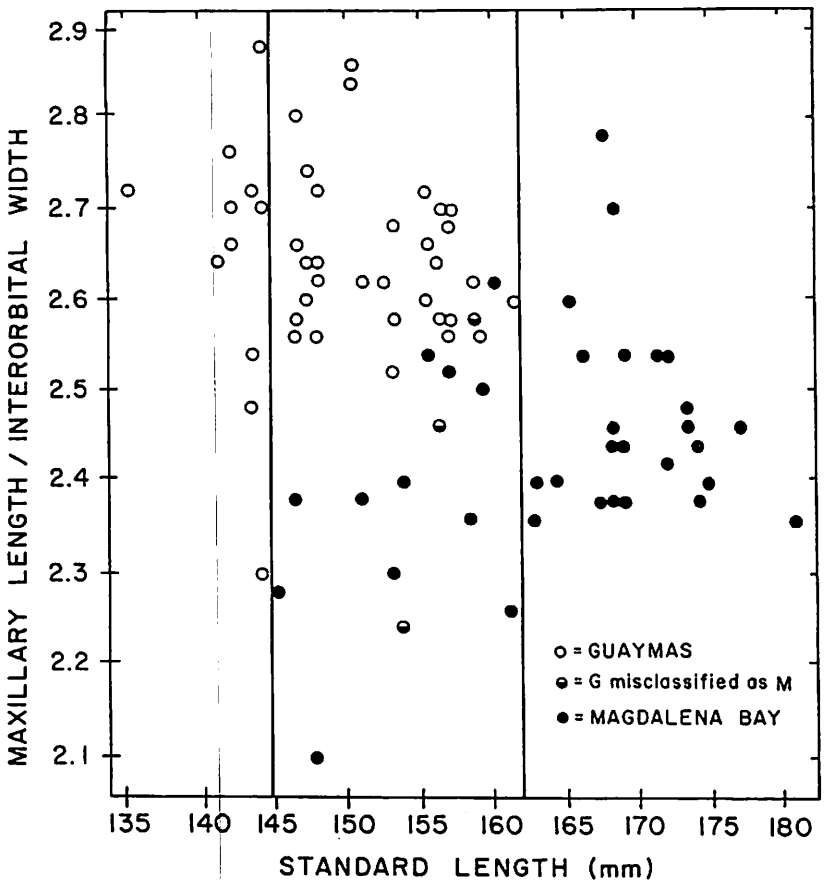

Figure 2. Samples of Pacific sardines collected off Mexico plotted to show patterns among three morphometric variables. Guaymas is on the Gulf of California and Magdalena Bay on the west coast of Baja California. The center panel of the graph contains data on fish that were sorted by a discriminant function analysis with only three misclassifications.

reported for historical sardine stocks. The differences in size at age apparently depend on where the fish are living. We can expect, therefore, that when the Pacific sardine regains its former abundance, yield characters for a re-established fishery in a particular area will be the same as they were formerly.

Cooperating Organizations

California Department of Fish and Game, Monterey and Long Beach
Centro Interdisciplinario de Ciencias Marinas, La Paz, Mexico
Departamento de Pesca, Guaymas, Sonora, Mexico
National Marine Fisheries Service, Southwest Fisheries Center, La Jolla and the Pacific Environmental Group, Monterey
Universidad Autonoma de Baja California Sur, La Paz, B.C.S. Mexico

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Chinook Salmon Spawning Behavior

Humboldt State University
R/F-103
Project Initiated: October 1, 1984
Project Completed: April 1, 1986

David G. Hankin

Project R/F-103 originated from an earlier Rapid Response project (R/NP-1-13A) of the same title. A no-cost extension for project R/F-103 was granted through March of 1986. This report covers two seasons (fall of 1984 and 1985) of behavioral observations of chinook salmon spawning behavior in Bogus Creek, a tributary to the Klamath River in northern California. The report is intended to serve as a final report for project R/F-103 and its extension.

Many fishery biologists have expressed concern that fishery-induced shifts toward smaller average sizes and younger ages in chinook spawning populations may result in long-term genetic shifts in maturation schedules of chinook salmon (Rutter, 1902; Ricker, 1980, 1981; Hankin and McKelvey, 1985; Healey, 1986; and others). The degree to which these concerns are justified may depend on the mating behaviors of chinook salmon. Age of maturation in chinook is known to have a strong heritability (Donaldson and Menasveta, 1961; Nicholas et al., 1984). If mating were random among spawning populations of smaller and younger chinook, then size-selective fisheries would result in long-term genetic shifts toward younger age of maturation. The overall objective of our research was to characterize the mating behavior of chinook salmon through statistically valid behavioral observations with emphasis on size dependence of mating success. Ultimately, information collected in this study can be used to evaluate the probability that fisheries have been responsible for genetic shifts toward younger age of maturation in chinook.

Methods

This project was designed specifically to test the following two null hypotheses: (1) male spawning success is independent of male size, and (2) female mate selection is independent of male size. Each of these null hypotheses was tested by relating the apparent spawning success and dominance status of males in their spawning associations with territorial females to the relative sizes of males and females.

As originally proposed, behavioral data were to have been generated from two sources: (1) observations of natural unmanipulated spawning aggregations, and (2) observations of manipulated spawning aggregations confined to experimental pens. Gravel enhancement activities at Bogus Creek in fall of 1984, at the proposed site of the experimental pen enclosures, ruled out the latter source of behavioral data, however. Indeed, observations of the spawning behavior of male chinook suggested that pen enclosures may not have proved successful (see below). Results presented in this report are therefore based entirely on behavioral observations of unmanipulated spawning aggregations.

Observations were made by the Sea Grant trainee (Randall Baxter) assisted by a Humboldt State University master's student in Wildlife (Lynn Roberts) during falls of 1984 and 1985. Methods used to collect behavioral observations consisted of a time- and area-stratified daily sampling regime following, for the most part, the focal female and point-sample approaches of Altmann (1974). Independent behavioral samples were collected by both observers throughout the 1984 and 1985 spawning seasons at Bogus Creek. Individual fish could be recognized by distinctive wounds, patches of fungus, fin erosion, and other characteristics of spawning chinook as their physical condition deteriorated. In addition, 163 and 285 fish were tagged with colored and numbered Peterson disk tags as they entered Bogus Creek in 1984 and 1985. Subsequent observations of these fish (while alive or as carcasses) allowed assessment of the reproductive lifespan of individual males and females, the dispersion pattern of fish within Bogus Creek, and estimation of the number of adults spawning below the Bogus Creek weir counting facility operated by California Dept. of Fish and Game.

Results

As a broad generalization, a typical spawning association consisted of a territorial female on her nest site with one or more males in association. A single male assumed dominance and maintained a position at the female's side by aggressively attacking and/or displaying to approaching males (subordinate satellite males). When a female was near spawning (release of eggs), subordinate males would take up positions downstream from the female paired with the dominant male and would "sneak in" at the time of spawning. Actual spawning acts (release and fertilization of eggs) took only a few seconds. Jacks (small, <60 cm, age two, precocious males) faced intense aggressive behavior from larger males and also from females. They failed to exhibit "challenging" behaviors (that larger and older males apparently adopted to establish dominance hierarchies) and exclusively used the "sneaker" strategy (see Gross, 1982).

Once having established a nest territory, females aggressively defended their nest sites against intruding females throughout their reproductive life until death. In contrast, males appeared to adopt a

"roving" habit. Males would court females, attempt to achieve a dominant position through aggressive encounters with other males, and would then "judge" whether a female was near spawning. If the female was not near spawning, the male would move upstream or downstream in search of a female that might be and would once more attempt to establish dominance, etc. Individual dominant males were observed to have spawned with (and presumably fertilized the eggs of) at least four different females.

Reproductive lifespans were more easily determined for females than for males because of their fidelity to their selected nest site. Forty females were observed from the time they had first begun nest construction until death in 1984, and 46 such females were observed in 1985; errors in calculation of reproductive life are probably ±1 day. Table 1 shows that average reproductive lifespan appeared to depend on time of entrance into Bogus Creek in both 1984 and 1985. Late-entering females had average life spans of about one day less than did early-entering females.

Table 1. Average Reproductive Lifespans and Average Number of Days Exhibiting Territorial Behavior among Female Chinook Salmon in Bogus Creek during 1984 and 1985.

Period	n	Mean Lifespan (s.d.)	Days Territorial (s.d.)
≤ 10/10/84	22	8.2 (2.12)	7.1 (1.89)
> 10/10/84	18	6.6 (2.05)	5.1 (1.46)
overall 84	40	7.5 (2.22)	6.6 (1.97)
≤ 10/08/85	21	9.4 (2.74)	8.2 (2.78)
> 10/08/85	25	8.3 (2.10)	6.5 (2.18)
overall 85	46	8.8 (2.45)	7.3 (2.59)

Reproductive lifespans for males appeared to be similar; but because males did not maintain fixed territories, calculated estimates were less reliable.

Seventeen actual (undisturbed) spawnings were observed during 1984 and an additional 16 were observed in 1985. Table 2 shows that most observed spawnings consisted of one or two males in association with a single female.

Table 2. Number of Males Associated with Females at the Time of Spawning in 1984 and 1985 at Bogus Creek. (Table Entries Give Number of Times Observed.)

	Number of Males in Spawning Aggregation							
Year	1	2	3	4	5	6	7	12
1984	5	6	1	3	2	0	1	0
1985	1	10	3	1	1	0	0	1

However, three or more males in association with a single female was not uncommon and for one observed spawning there were approximately 12 males participating. The dominant (alpha) male was the first (or "even") to fertilize eggs in all observed spawnings in 1984, and was the first (or "even") in eight out of ten cases where two males were involved in 1985. In only two of ten cases in 1985 were jacks one of the two males involved, and in both cases they did not successfuly participate in spawning. When more than two males were associated with a female, the dominant male always spawned and was usually, but not always, first. Satellite males usually spawned on the opposite side of the female from the dominant male.

Sizes at age for Klamath River male and female chinook are comparable until ages five or six (Snyder, 1981), and most Bogus Creek fish were ages two to four. Because maturation schedules for males are earlier than for females (i.e., males first mature at age two, whereas females first mature at age three), average sizes of males were less than average sizes of females among the total Bogus Creek spawning population. Table 3 shows, however, that dominant males were always at least as large as spawning females. In most cases, dominant males were substantially larger than females.

Table 3. Relative Sizes of Dominant Males Compared to Females in Observed Spawnings during 1984 and 1985 at Bogus Creek. (Table Entries Give Number of Times Observed.)

	Relative Size of Dominant Male				
Year	<	≤	≈	≥	>
1984	0	0	5	5	8
1985	0	0	3	2	11

Discussion

Behavioral observations made during this research strongly support a hypothesis that larger and older male chinook salmon participate in spawning to a far greater degree than would be suggested by their relative abundance on spawning grounds. Larger males could more easily establish dominance among other males, dominant males paired with females were always at least as large as females, dominant males were nearly always first or "even" at actual spawning, and single dominant males were observed spawning with up to four females.

Schroder (1981) demonstrated a strong, if imperfect, relation between actual fertilization success of male chum salmon and visual observations that males had spawned first and/or had excluded other males. Based on our behavioral observations and Schroder's work, the implications are that older and larger male chinook salmon contribute far more genetic material to the next generation than would be the case if mating were random with respect to size. In this manner, the mating behaviors of chinook salmon may buffer and/or ameliorate the long-term genetic shifts in age of maturation that might otherwise result from size-selective fisheries. The degree to which these behaviors can buffer shifts in age of maturation must, of course, also depend on the sizes and ages of fish that actually reach spawning grounds. Intensive offshore troll fisheries on Klamath River (Bogus Creek) fish have effectively eliminated six-year-old males from spawning runs and have

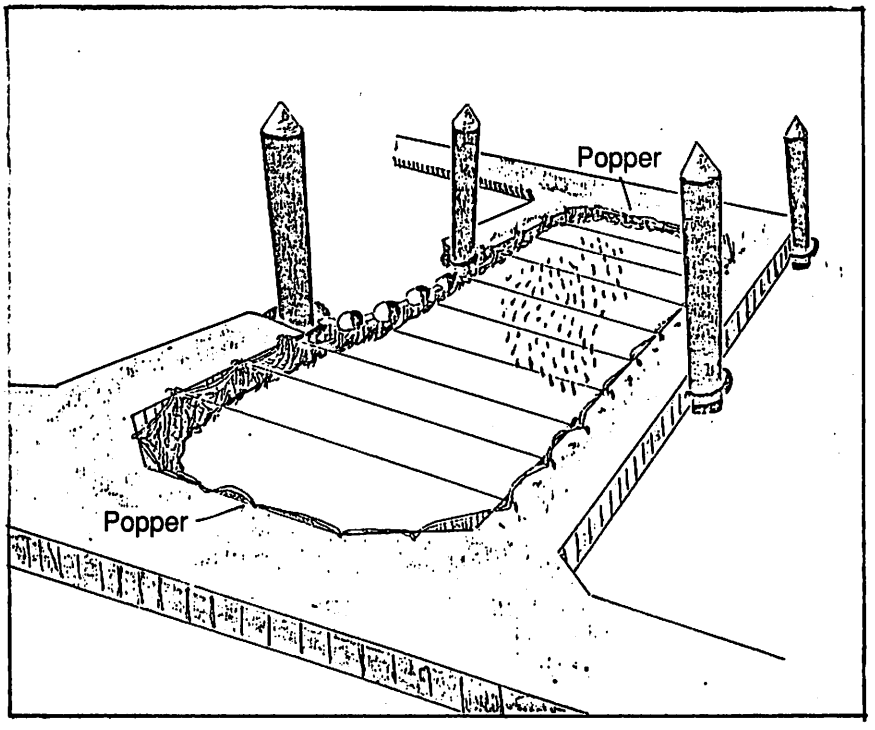

Figure 1. Schematic drawing of experimental site at J-Dock, Santa Cruz Harbor.

inherent in the less-direct indices such as sonar or gill nets possible at a power plant intake or in the harbor mouth, and in an unconfined environment there is no practical way to monitor habituation of the fish to stimuli. Also, we are interested in detailed analysis of both individual and school response. An acoustically benign experimental environment makes possible controlled experiments and data gathering impossible in either the lab or the field.

It was hoped that doing the tests in this harbor environment would solve or at least minimize the reflection and reverberation problems inherent in acoustic work in tanks. Tank walls constitute effective acoustic mirrors and create complex sound fields with inconsistent amplitude decay and reduced directionality. These potentially confounding factors are often neglected in bioacoustic studies (Hawkins, 1986).

The J-Dock experimental environment was meant to provide a combination of the direct observation possible in a laboratory and the more benign acoustic environment in the harbor. The gradually sloping harbor banks and mud bottom effectively reduced or eliminated the standing waves that limit directionality to the first transient of sound in a conventional tank.

In the course of the work considerable difficulties were encountered with the physical environment. First, surge conditions at J-Dock were much more severe than we had anticipated. Maintenance of the enclosure was a struggle since it was too large to be maintained comfortably with the materials and resources available.

The acoustic environment was also less benign than anticipated. As we made and began to analyze sound recordings we saw that there were three acoustic problems, one with the site and two with the equipment.

First, the sounds were above the amplitude range of the hydrophone, and we saw seriously clipped signals (Figure 3). Also, the tape recorder was producing unreliable measures of amplitude due to its imprecise gain control. These difficulties were relatively easy to resolve with more appropriate gear.

The other, and in the long term more profound, acoustic difficulty is one of sound transmission through the J-Dock environment. As a result of the very shallow water depth at the test site, a persistent acoustic phenomenon called "wave guide" was present (Urick, 1967). In a wave guide, the dimensions of the propagating medium, in this case water, limit the magnitude of the wave length that will propagate. Simply put, the 2½ to 3 m depth at J-Dock was not deep enough to carry the sound more than about three meters. Farther from the source most of the low-frequency energy was lost (Figure 4). Note the abrupt loss of the fundamental (60 Hz) component. It is to this low-frequency sound that fish are expected to react (Tavolga et al., 1982).

We are still able to demonstrate that, within the unrestricted sound field, both croakers and anchovies react strongly to both sound sources. When one of the devices fired, the typical response of nearby fish was a "startle" reaction, followed by swimming away from the sound source. This reaction falls off rapidly at the same distance that the sound field is distorted by the wave guide.

The waveguide prevented propagation beyond 3 m at J-Dock. The potential radius of influence, which may be more that 3 m, could not be directly observed at this location. A more exact estimate, if made directly, would require deeper water at the test site. Recordings made in the harbor mouth indicate that the same waveguide phenomenon (an abrupt loss of low-frequency component) occurs there. As a result of the greater (about 8 m) water depth, the low-frequency component reduction occurs at a distance of about 16 m from the source. Therefore, the range of the sound's effect on fish was at least 3 m at J-Dock, and in the harbor mouth would probably not be more than 16 m. This upper limit is set by the water depth in the harbor mouth, and within this limit the attenuation of the sound with distance must be

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dramatically reduced the number of five-year-old fish. Whether or not these reduced numbers of larger older fish with their apparent enhanced mating success can prevent gradual shifts in age of maturation must remain an open and important question in fisheries management.

Cooperating Organizations

California Department of Fish and Game, Anadromous Fisheries Branch
Iron Gate Hatchery

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Schooling Behavior of Anchovies

Kenneth S. Norris

University of California, Santa Cruz
R/F-108
Project Initiated: October 1, 1985
Project Completed: September 30, 1986

This work addresses two closely related but distinct lines of research. On the one hand we seek to investigate the phenomenon of sensory integration in schools of fish, primarily northern anchovy (*Engraulis mordax*). Although much is known about the form and function of fish schools (for a current review see Pitcher, 1986), the mechanisms by which they operate remain more obscure.

Our other and more immediately practical goal is to help develop an effective, economic, and environmentally benign fish control system. The current work involves the use of high-amplitude low-frequency sound as a deterrent of schooling fishes.

An effective behavioral fish-control system would be useful to the operators of dams, pump stations, power plants, and harbors. The Santa Cruz, California, harbor has experienced massive anchovy kills that are expensive, ecologically damaging, and unpleasant. There have been four serious anchovy kills since the harbor opened in 1964.

Utilities spend large sums building and maintaining screening systems with limited success. Fish loss to power and irrigation systems is widespread in both fresh and saltwater (ESEERCO, 1985), and the electric energy generating industry is actively conducting research to reduce fish mortality at its cooling water intakes (EPRI 1984). Impingement at power plants and water diversion facilities result in significant losses of striped bass (*Morone saxatilis*) in shallow inland waterways in California (Chadwick, 1979).

Sound offers several advantages over other potential behavioral deterrents. Sound propagates well in water, and sound stimuli—unlike light—are not seriously affected by turbidity, biofouling, or ambient light conditions. Sound is safer and easier to produce in water than is an electric field. Sound is important in the aquatic environment, and fish are well adapted to transduce and react to sound (Tavolga et al., 1982).

In industrial research, the work of Dr. Paul Patrick and colleagues at Ontario Hydro (a Canadian utility) is exemplary. They tested sound sources built by Bolt Technologies (a Norwalk, Connecticut, company that supplies the oil exploration industry with sound sources) at power plants in Canada. Using sonar and gill nets to assay fish response they found significant (71%-99% for periods of up to six hours) reduction of numbers of alewife (*Alosa pseudoharengus*) from an experimental structure in a power plant intake channel. They ran tests with arrays of up to 12 of Bolt's sound sources (Haymes and Patrick, 1986).

The Bolt system uses high (3000 psi) pressure to make sound in two different ways: the "air gun" or "popper" releases air suddenly and makes a very loud thump at 60 cycles (Hertz). The other device, a "water gun," uses the same air pressure to drive a piston, which creates a cavitation. This evacuated space in the water slaps shut with a much louder broad-band crack from approximately 50 Hz to approximately 10 kHz.

With funding from Southern California Edison, we leased the Bolt Technologies system to conduct tests on white croaker (*Genyonemus lineatus*) and northern anchovy. Croakers are a problem at Southern California power plants, and anchovies are of interest to both the utility and the Santa Cruz Port District. The Port District provided the site and considerable material and logistic assistance.

The study was timed so that the sound equipment was on hand for the late summer anchovy peak when kills are most likely, and during the most critical period the equipment was deployed in the Santa Cruz harbor mouth. This was done both as a service to the Port District and to provide for a possible field test of the equipment. There was no opportunity for such a field test since there was no incursion of large numbers of fish this year (1986).

The experiments we conducted were designed to test the extent (radius of influence) to which these devices will keep fish away. The original experimental design called for fish schools to be enclosed in a net hung from J-Dock in the upper Santa Cruz harbor. Unfortunately, the opacity of the water was such that the net had to be lined with polyethelene sheeting so that the water could be filtered for clarity. The enclosure was 32 meters long, 7½ meters wide, and about 1 meter deep (Figures 1, 2).

The polyethylene liner, because of its extreme thinness (6 mill) relative to the wavelengths in question (over 100 ft for 60 Hz) and its density (only slightly less dense than seawater), was effectively transparent to the sound.

The sound sources were fired outside the liner to eliminate nonacoustic (especially visual) stimuli. The air gun was fired every 4 seconds for comparability with the Canadian work. The water gun was fired every 15 seconds because of its more involved and depth-dependent hydrostatic cocking mechanism. The Bolt firing circuit was triggered with a Potter relay. Water clarity was maintained by recirculating through a sand pressure filter.

We believe that there is no substitute for the direct observation and recording of fish reaction. There is unavoidable ambiguity

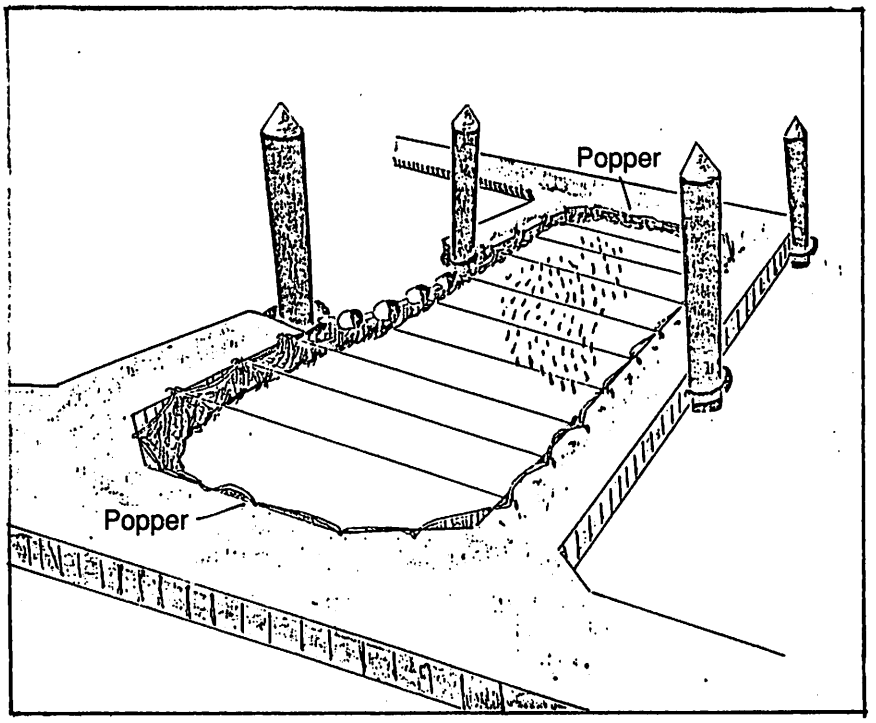

Figure 1. Schematic drawing of experimental site at J-Dock, Santa Cruz Harbor.

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The waveguide prevented propagation beyond 3 m at J-Dock. The potential radius of influence, which may be more that 3 m, could not be directly observed at this location. A more exact estimate, if made directly, would require deeper water at the test site. Recordings made in the harbor mouth indicate that the same waveguide phenomenon (an abrupt loss of low-frequency component) occurs there. As a result of the greater (about 8 m) water depth, the low-frequency component reduction occurs at a distance of about 16 m from the source. Therefore, the range of the sound's effect on fish was at least 3 m at J-Dock, and in the harbor mouth would probably not be more than 16 m. This upper limit is set by the water depth in the harbor mouth, and within this limit the attenuation of the sound with distance must be

considered.

The maximum possible effective radius of influence varies directly with water depth. The depth at the San Onofre Nuclear Generating Station intake is approximately 10 m (Dr. Terry Sciarrotta, Southern California Edison, pers. comm.). Deeper-water experiments in a similar (but smaller) enclosure in future work will extend the radius of unrestricted sound.

In an attempt to arrive at a less direct but more useful result we have recorded fish response and the sound field at lower air pressures and therefore lower amplitudes. Tests were conducted on both species and with both devices with in-line air pressure (to vary amplitude) at 500 psi intervals from 500 psi to 3000 psi and also at 250 psi. Fish response was recorded on videotape, which also records for reference the sounds of the Bolt devices.

A separate acoustic tape was made with a Racal 4 DS 4-channel tape recorder (with FM channel for low frequencies) and a Bruel and Kjaer 8103 hydrophone through a Bruel and Kjaer 3624 amplifer. Sounds were recorded at half-meter intervals away from the sound sources to a distance of 5 m and at 8 and 16 m.

The majority of these recordings await analysis, but absolute peak-to-peak amplitudes of both devices at 3000 psi from 1 meter away have been determined as 197 dB for the air gun and 216 dB for the water gun, each referred to a micropascal at 1 m.

If we can determine that fish invade the 3 m range of undistorted sound, we may be able to arrive at a critical sound-amplitude estimate in the restricted acoustic environment that would yield a calculated range of influence for the devices at full amplitude in an unrestricted acoustic environment. It will be early March 1987 before the analysis is far enough along to know if a better range estimate is possible.

Most promising are the indications of duration of response. Although the croakers seem to habituate

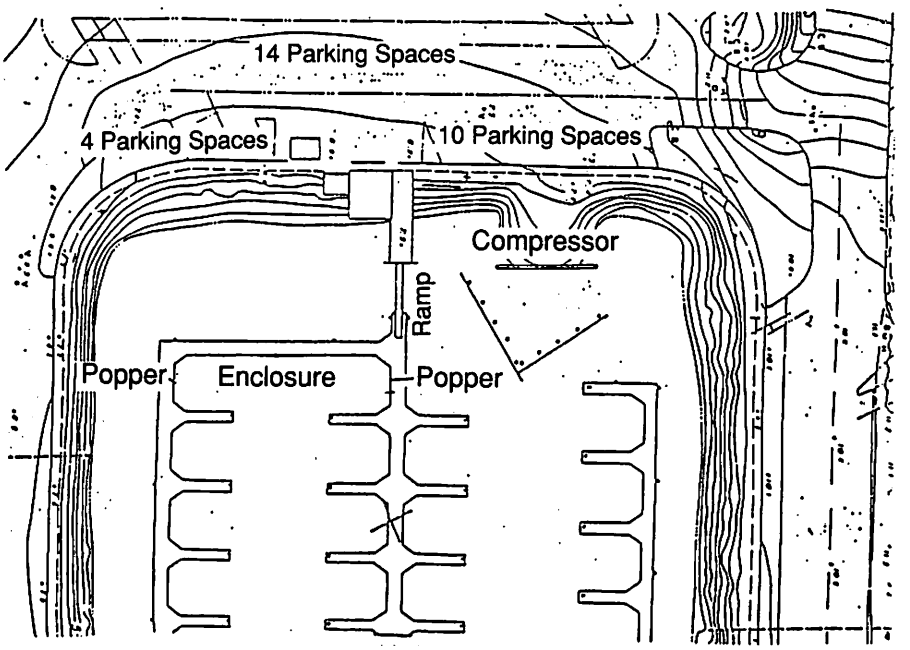

Figure 2. Location at "J-Dock", Santa Cruz harbor.

within minutes, the anchovies showed no obvious reduction in response for up to 5 hours. With the air gun, the fish within the unrestricted sound field showed a marked "startle" response followed by swimming away from the sound. The water gun, which is much louder, caused a more extreme but similar response.

The fish did not respond equally to every firing of the air gun, which was set to fire on a 4-second interval. Whether this was due to inconsistency of the sound source is unknown now, but analysis of the videotape sound track will address this question. The anchovies consistently responded strongly to the water gun, which fired every 15 seconds.

Croakers habituate (or learn) fairly quickly, and the anchovies do not. The longest test of the air gun on anchovies was about 5 hours, and the longest with the water gun was about 3 hours. Preliminary analysis indicates no reduction in response for the anchovies during any test. Firing the air gun every 4 seconds for 5 hours amounts to 4500 firings. We submit that this extreme resistance to habituation and extinction of response is remarkable in any animal and in this case may provide a key to both improved fish-control systems and a better understanding of the way fish schools work.

Since they showed no evidence of habituation to either device, it is not surprising that the anchovies did not learn to stay away from the area of highest sound intensity. They would swim away when the device fired, but before the next firing (either 4 or 15 seconds later, depending on the device used) would resume "normal" behavior in that their school direction had no consistent orientation relative to the sound source. This indicates that repetition rate may be an important variable in fish control and may need to be minimized for best results. The effect of repetition rate warrants further study.

There are at least two reasons that anchovy schools might not habituate while croakers do. Both are derived from the differences in behavioral ecology of the two species and are not mutually exclusive.

The first deals with the difference in school function and structure between the two fishes. The croaker is a demersal facultative schooler that orients to the bottom as well as to the school. Croakers are creatures of a substrate,

whereas the pelagic and obligatorily schooling anchovies live exclusively in the ever-changing world of the school. The functioning of the school requires high levels of coordination, and any process that would change some members' response relative to others would reduce the level of integration and subsequent effectiveness of the school. Since a well-integrated and effective school is crucial for survival, it may be that consummate schoolers such as anchovies have adaptively reduced their susceptibility to habituation. For anchovies (and not for croakers) learning may be contrary to the function of the school.

On the other hand, it may be that it is specifically high-amplitude low-frequency sound to which anchovies resist habituation. Sound is a traveling wave of the compression and rarefaction of a medium as well as the concomitant particle displacement (Hawkins, 1986). The lateral line is capable of transducing both aspects of sound as well as the displacements produced by the swimming motions of neighbors in the school (Tavolga et al. 1982).

Near a high-amplitude low-frequency sound source the particle displacement of the sound might be interpreted by the fish as if from a neighbor's movement. It is this sort of stimulus that, along with vision (Pitcher, 1986), integrates fish schools. If high-amplitude low-frequency sound is affecting the schools' sensory integration system via the lateral line, it is not surprising that the fish do not habituate (Blaxter and Batty, 1985).

We are in the process of analyzing our summer's work and will continue to report our results to California Sea Grant, Southern California Edison, and the Santa Cruz Port District. Thus far only the most cursory analysis of the sound and videotapes has been done. Analysis of the tests of different air pressures may provide an estimate of critical amplitude for the two devices and the two species.

We hope to continue this project next year on a smaller scale and in deeper water. In the interim, experiments with non-acoustic stimuli in tanks will be undertaken to determine if anchovies resist habituation of other sensory modalities.

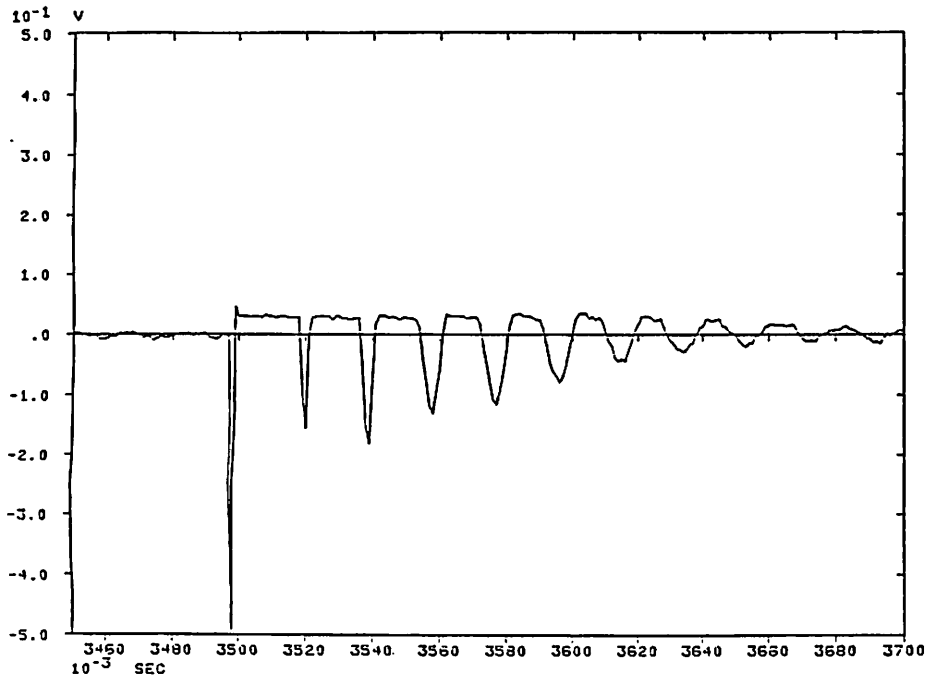

Figure 3. "Clipped" signal showing overload in Magnavox hydrophone preamplifier.

This work is encouraging in that it indicates that high-amplitude low-frequency sounds can be an effective and persistent behavioral deterrent for schooling fish. Results are comparable to the work of others in the field and will be useful to them. This kind of study is directly applicable to industrial use in the field (Paul Patrick, Ontario Hydro, pers. comm.). We look forward to continued cooperation between the Santa Cruz Port District, the Utilities, California Sea Grant, and the Marine Acoustic Services Laboratory.

Cooperating Organizations
Lockheed Missiles and Space Company, Santa Cruz
Santa Cruz Port District
Southern California Edison

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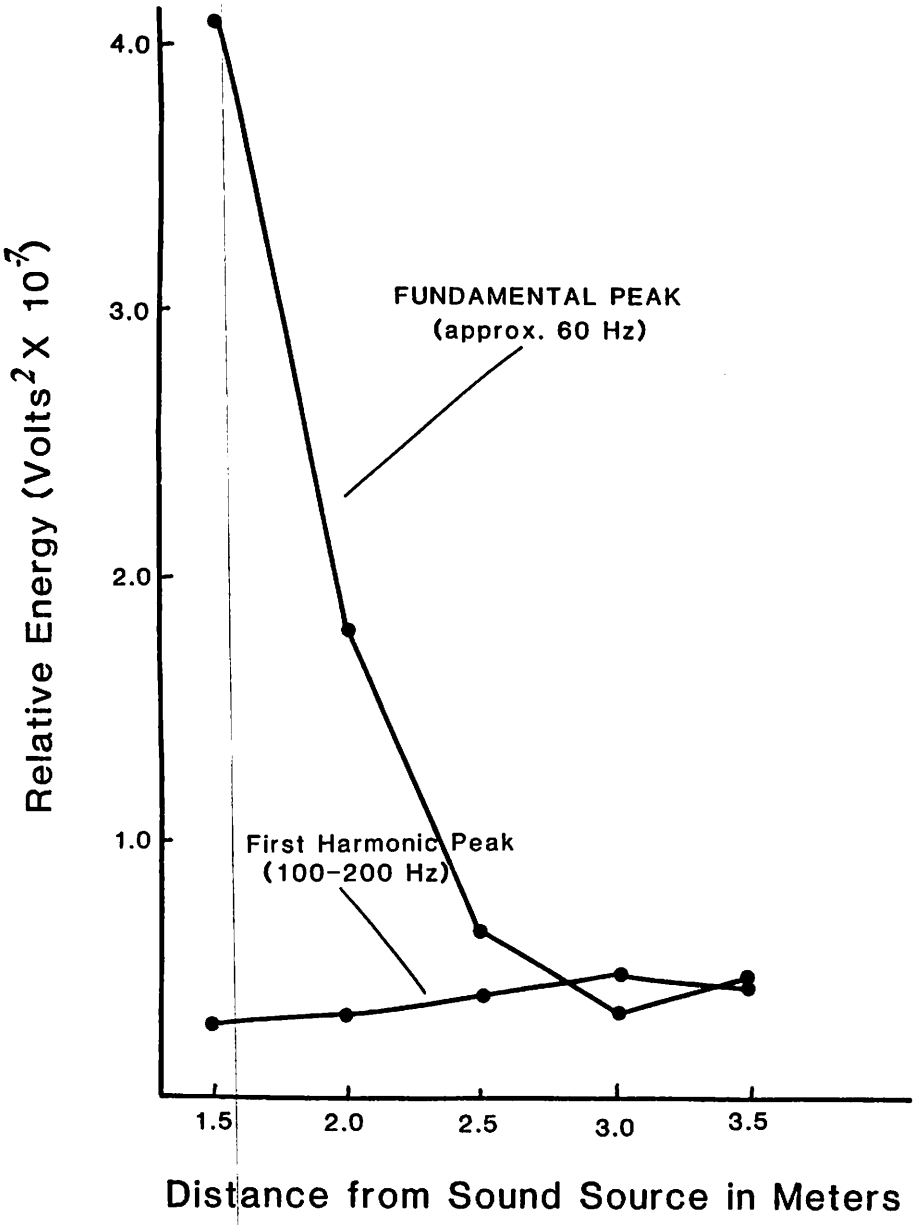

Figure 4. Comparison of energy decay over distance for high- and low-frequency components of "popper" sound at J-dock site.

Brood Mortality and Egg Predation in King Crab Fisheries

University of California, Santa Barbara
R/F-AL
Project Initiated: October 1, 1983
Project Completed: August 12, 1985

Daniel E. Wickham and Armand M. Kuris

The study of field mortality of eggs of Alaskan king crabs is progressing along five separate avenues: (1) survey of mortality to various king crab species, (2) geographic survey of mortality to the red king crab (*Paralithodes camtschatica*), (3) seasonal cycle of egg mortality to *P. camtschatica*, (4) taxonomic identification of egg mortality agents and symbionts of Alaskan king crab hosts, and (5) reconstruction of historical data which may apply to the problem of egg loss in Alaskan waters.

King Crab Species

So far the red king crab, *Paralithodes camtschatica*, appears to be the only king crab species suffering from significant egg mortality. The golden or brown king crab, *Lithodes aequispina*, from several regions appears to be nearly free of egg clutch symbionts. Nemertean egg predator worms are occasionally found on *L. aequispina* but only in low numbers, and egg mortality in the collected samples presently is negligible.

Egg mortality and infestation by egg predators on blue king crabs (*Paralithodes platypus*) are also limited in extent. Egg mortality in this species appears to be virtually absent. One species of crab that was incidentally sampled in connection with the present study showed extensive egg loss resulting from a massive infestation by nemerteans in certain regions. This is the Tanner crab, *Chionoecetes bairdi*, which appears to be infested by the same species of nemertean found on red king crabs.

Geographic Survey

Two regions, characterized by low prevalence of egg predators and minimal egg mortality in one, and high prevalence of egg predators with extensive egg mortality in the other, can be discerned in the samples analyzed to date. The region extending from the Alaskan peninsula west through the Aleutians and north into Bristol Bay and the rest of the Bering Sea typically displays low prevalences of egg predators. In these populations measurable mortality to the eggs is very low and egg survival appears to be quite good.

The central and eastern Gulf of Alaska, beginning with locations around Kodiak Island and extending into Kachemak Bay inside Cook Inlet and down to the Southeast region of the Alaskan Panhandle, are typified by populations of crabs harboring massive infestations by a species of nemertean that can cause the total destruction of eggs in several of the populations sampled.

A detailed study of small-scale geographic variation between the crab "schools" around Kodiak is underway so mortality in individual subpopulations can be integrated with crab distribution and abundance to arrive at an overall regional estimate of mortality.

Seasonal Variation

Repeat sampling in both Kachemak Bay and around Kodiak has helped us to construct a tentative life cycle for the nemertean species found in high numbers on crabs from these areas. Samples taken from early egg broods in the months of March, April, and May are generally free from nemertean infestations, and egg mortality is virtually absent. During June and July massive infestations of nemerteans suddenly appear and begin preying on host eggs. Samples taken when the nemerteans first appear begin to show egg destruction. The egg loss accumulates through the summer and in certain populations around Kodiak and in the Kachemak Bay population egg loss is total by August or September.

These observations are crucial to our understanding of the studies necessary to assess field mortality accurately. The red king crab broods its egg clutch for approximately 11 months beginning generally around March. Surveys conducted prior to June would show no evidence of egg loss even in a population that was at serious risk. Samples taken from June through August would demonstrate mortality as well as contain the agent of the mortality. Samples taken from October until hatch the following February would display egg losses. But even in heavily infested individuals we have found that the worms disappear from the host. Thus, attribution of egg loss to a particular course in such late season samples would be impossible without previous knowledge of infestation rates.

One other important aspect of the seasonal observations is the demonstration that all eggs collected from March through June were fertile and developing normally without mortality. Predation by nemerteans appears to be the only significant source of egg loss. Observations in the laboratory by A. J. Paul (personal communication) have demonstrated the role of this particular nemertean in host egg mortality and confirmed our field observations. Earlier researchers had suspected that egg loss in the red king crab was due to insufficient mating activity by male crabs, or insufficient numbers of males. Eggs would be extruded unfertilized and then sloughed off. Our observations demonstrate that at this time there appears to be a very high rate of fertilization. Mortality occurs later,

during embryonic development, and is predominantly the result of predation by nemerteans.

Taxonomic Studies

The complex of nemerteans found in Alaskan waters has caused us to completely revise our thinking about the genus *Carcinonemertes*. At this time it appears that at least three (possibly five) distinct nemertean species occur on king crabs.

Our initial observations from heavily infested populations in the Southeast, Kachemak Bay, and Kodiak implicated a very small worm in the genus *Carcinonemertes*, maturing at a length of as little as 1 mm. It possesses the normal complement of 2 eyespots and resembles the species *C. errans* in most respects except for a significant difference in size. A. J. Paul (personal communication) observed that the larval stage of this worm was a "pilidium" larva. This is a distinctive helmet-shaped larva, unique to the phylum Nemertea. The genus *Carcinonemertes*, however, does not possess such a larva. The larva of other *Carcinonemertes* spp. is a simple ciliated ellipse. The observation of a pilidium larva suggests that the species causing the heavy mortality of red king crab eggs may not be assignable to the genus. Even if this form is still best placed in the genus, it is clearly a new species.

Samples taken in the Aleutians display the presence of a very large (up to 30 mm long) eyeless nemertean egg predator. The numbers of this form are generally low, but certain crab egg clutches contained several hundred of these worms and could lose as many as one-quarter of their eggs. Observations on sectioned specimens demonstrated that this nemertean's mouth was "subterminal." All other members of the genus *Carcinonemertes* have a common mouth and proboscis opening at the terminus of the anterior end of the worm's head. This species is the first nemertean egg predator with a subterminal mouth and what appears to be a separate, terminal opening of the proboscis. Again it is unclear whether this worm can be reliably placed in the genus *Carcinonemertes*.

Another eyeless form co-occurs with the small 2-eyed species in the Gulf of Alaska. This form is intermediate in size but posesses a unique internal feature. To date, all members of the genus *Carcinonemertes* have been found to contain a proboscis that is armed with a basis that has a single stylet attached to it. No accessory stylets have ever been observed in the genus *Carcinonemertes*, a generic feature unique among the hoplonemerteans. The eyeless form found in the Gulf of Alaska has two accessory stylet pouches present, each containing generally two accessory stylets. The only other nemertean crustacean egg predator with accessory stylets is the 4-eyed *Pseudocarcinonemertes homari*, which is found on the American lobster (*Homarus americanus*). Again generic placement of this eyeless species awaits further study.

One specimen of an unusual 2-eyed nemertean that had an expanded head was seen in living material at Seward, but so far we have not seen any more individuals of this form.

The small 2-eyed worm and the intermediate eyeless worm with accessory stylets are both found not only co-occurring on *P. camtschatica* but also appear to be on the Tanner crab *Chionoecetes bairdi*. The 2-eyed worm has been seen in great numbers on Kachemak Bay Tanner crabs where it causes extensive egg mortality, as well as in high numbers in some locations around Kodiak. A complete survey of Tanner crabs is not within the scope of our study, but the appearance of regions with extensive egg loss coupled with recent declines in that fishery argue for the expansion of our studies on egg mortality to include this species.

Tag Return Analysis of California Sturgeons and Elasmobranchs: Technique Verification and Transfer

Moss Landing Marine Laboratories
A/S-2
Project Initiated: October 1, 1985
Project Completed: September 30, 1986

Gregor M. Cailliet

Over the past several years, much attention has centered on California sturgeons and elasmobranchs, primarily because of their increasing popularity as targets for fisheries and because of those life history features that make them particularly susceptible to these fishing pressures. For some species, well-documented information on such parameters as age, growth, reproductive habits, and migration patterns has resulted. In others, critical information is still missing.

For white sturgeon, much work has centered on age and growth, reproduction, physiology, and population dynamics based on catch statistics and size composition (Kohlhorst, Miller, and Orsi, 1980; Miller, 1972a, 1972b). Presently, researchers at the University of California, (UCD) Davis are continuing to work on these subjects in cooperation with the California Department of Fish and Game (CDF&G) and our researchers from Moss Landing Marine Laboratories. A tagging program has also been part of this cooperative research effort with approximately 1400 sturgeon captured, measured, tagged, injected with oxytetracycline (OTC), and released in San Pablo Bay for verification of age estimates from tag returns.

Similarly, much research has centered on the ecology of elasmobranchs (Cailliet and Bedford, 1983; Cailliet et al., 1983a, 1983b), yet little information is available that can be used to verify age determinations in most species. Recently several projects have been initiated to tag three species of pelagic sharks and several additional species of nearshore demersal sharks. Laboratory analysis of hard parts from returned sharks is the only valid means of providing field verification of growth rates and information related to stock structure and migration patterns.

To properly manage a fishery, it is essential to have accurate and precise methods of ageing. Therefore, the overall project objectives were (1) to continue our field tagging activities, (2) to aid ongoing field tagging programs, (3) to collect specimens from these tag-recapture studies, (4) to analyze hard parts of tag returns to assess field growth, (5) to evaluate periodicity of growth-band formation, and (6) to transfer our technical abilities to personnel of CDF&G through cooperative research activities.

Over the past six years under Sea Grant support, we have been studying age and growth characteristics of elasmobranchs, sturgeon, and billfishes using calcified growth bands in vertebral centra (Cailliet et al., 1981, 1983a, 1983b; Cailliet, Radtke, and Welden, 1986; Natanson, Cailliet, and Welden, 1984; Cailliet and Radtke, 1987) and other hard parts. During this period, vertebrae, body size measurements, and information on reproduction have been taken from sharks, sturgeon, and billfishes collected from commercial and sport fishermen and seafood processors. We have used several procedures to delineate growth bands in 22 species of elasmobranchs, 2 species of sturgeon, and 1 species of billfish. Techniques used to determine the periodicity with which these growth bands are deposited include histological analysis of centrum characteristics over time, tetracycline injections to mark calcifying zones, and radiometric dating techniques (Welden et al., 1987).

The importance of sturgeon age determination in fishery management is evidenced by the numerous and various methods used by different authors dating back to the early 1900s (Cuerrier, 1951; Kohlhorst, Miller, and Orsi, 1980). We have used various hard parts, including scutes, pectoral fin rays, otoliths, and other skeletal hard parts, to assess age of sturgeon in California. However, validation of sturgeon ages has never been considered. Use of inaccurate ages can cause serious errors in the management and understanding of fish populations (Beamish and McFarlane, 1983). Only by mark-recapture studies or use of known-aged fish can all age classes in a population be validated.

In our elasmobranch age-determination studies, we have made progress verifying the temporal nature of the growth rings using lab grow-outs and histological analysis, radiometric dating, and electron microprobe analysis of the vertebrae (Cailliet, Radtke, and Welden, 1986; Cailliet and Radtke, 1987; Welden et al., 1987). However, the best indicator of the temporal pattern of band deposition is field-tagged, tetracycline-injected specimens that have been recaptured (Beamish and McFarlane, 1983), so that growth subsequent to the tetracycline deposition zone can be evaluated. Existing cooperative tag-recapture efforts have supplied, and continue to supply, valuable samples for assessing periodicity of band formation and for more accurate and reliable age estimates.

In cooperation with UCD and CDF&G, we have tagged and injected tetracycline into approximately 1400 of the approximately 5000 sturgeon

collected, tagged, and released by the CDF&G during September and October of 1984 and 1985 in the San Pablo Bay, California. Sturgeon were collected using a 600-fathom trammel net and were double tagged with disk dangler tags. To date, an impressive number of recaptures (184) have been reported, 66 of which had the head and pectoral girdles saved for age analysis. In all of these, the tetracycline injection resulted in a very noticeable fluorescent mark in all hard parts illuminated with ultraviolet light. Thus, we have already accumulated a substantial number of samples for growth and age verification.

Pectoral fin rays were removed, dried, and cross-sectioned, and rings have been counted subsequent to the tetracycline mark. We have performed a similar analysis of rings in otoliths and the other bony parts collected. The hypothesis tested was that one pair of translucent and opaque bands is deposited each year. Only 9 of these samples have been at large for more than one year, and analysis of these few samples appears to support our hypothesis (see Figure 1).

There are several ongoing elasmobranch tagging projects now centering primarily on commercially important species. Dennis Bedford, CDF&G, Long Beach, has organized a volunteer tagging program in southern California for pelagic sharks and has tagged and released 256 sharks (206 blues, 20 threshers, and 30 bonitos). Of these, 80 were also injected with tetracycline. To date, 5 tagged sharks have been returned (3, 1, and 1 of each species, respectively), none of which had been injected with tetracycline. Dr. Milton Love, University of California, Santa Barbara, has tagged, injected, and released 130 Pacific angel sharks, 6 of which have been recovered after being at large for up to 3.5 years. A central California volunteer, Dan Livingston, has tagged and injected 27 bat rays, 15 leopard sharks, 2 sevengill sharks, 2 big skates, and 1 spiny dogfish in San Francisco Bay and Elkhorn Slough, and has tagged and measured 8 blue sharks in Monterey Bay. In addition, we have tagged 25 angel sharks, 22 bat rays, and several leopard, smoothhound, and sevengill sharks.

We have analyzed ring deposition in vertebrae prepared following Cailliet et al. (1981, 1983a), and growth subsequent to the

Figure 1. Section of the pectoral fin ray from a white sturgeon that was tagged and injected with oxytetracycline on September 20, 1984 and recaptured on December 20, 1986. This fish grew from 105 to 119 cm TL. Note the fluorescent band, which is the oxytetracycline (OTC) under ultraviolet light, and the new growth outside of this fluorescent band (marked by the arrow).

tetracycline mark has been evaluated using methods described by Smith (1984) and Cailliet, Radtke, and Welden, (1986). The hypothesis tested for each species of elasmobranch involved was that one pair of translucent and opaque bands is deposited per year in each hard part. Material from 16 tag returns has enabled us to test this hypothesis for two California species (6 angel sharks and 10 leopard sharks). For leopard sharks, we have further validated the annual nature of growth-band formation (after Smith, 1984). Angel sharks do not deposit their growth bands annually (see also Natanson, Cailliet, and Welden, 1984). We have also analyzed vertebrae from 1 whale shark that had been marked with tetracycline and held in captivity for almost two years at the Okinawa Aquarium, Japan, and its growth zones were annual. In addition, a total of 139 specimens of brown and gray smoothhound sharks were aged using X-radiography to elucidate growth rings in cross-sectioned vertebral centra. Deposition of translucent and opaque bands appear to be seasonally influenced and annual.

Cooperating Organizations

California Department of Fish and Game
University of California, Davis
University of California, Santa Barbara

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New Marine Products

Blank

Natural Marine Products That Regulate Mineral and Bone Metabolism

University of California, San Diego
R/MP-28
Project Initiated: October 1, 1982
Project Completed: September 30, 1985

Leonard J. Deftos

Marine species offer unique opportunities (1) to identify novel materials that affect mineral and bone metabolism and (2) to determine mechanisms of action not possible in more conventional, and more complicated, mammalian models of the skeleton. Precedent for the former was established with the discovery of salmon calcitonin and its clinical use in patients with metabolic bone disease. The reasons for the second are several: Shark's skeletons are composed primarily of cartilage, not bone; the skeletal system is thus more simple and homogeneous than in other species. In sharks and fish, calcitonin is produced in a discrete organ, the ultimobranchial gland, making ablative surgery feasible, in contrast to the case in higher species where calcitonin-synthesizing cells are scattered throughout the thyroid gland and elsewhere. No evidence has been presented that there are parathyroid hormone (PTH) glands in species more primitive than amphibia; it is thus simpler to evaluate effects of hormones and drugs without secondary, complicating PTH changes. Our preliminary data show the convenience of comparative (shark vs. bony fish) studies of dramatic changes in mineral metabolism.

After detailed anatomical studies, we have chosen to focus on the hornshark (Heterodontus) for development of techniques for hypophysectomy (pituitary) and epiphysectomy (pineal body). This species is readily available in waters off the Southern California coast, and we are encouraged by its anatomical simplicity. Other workers have described the importance of the pituitary in mineral regulation. All our experiments will be repeated in hypophysectomized and epiphysectomized sharks.

Striking differences have been noted in our results to date and represent differences in the two classes of vertebrates, chondrichthyes and osteichthyes. The sharks' sera are isomotic with respect to seawater, urea being the major osmotic contributor. Serum protein levels of sharks are 40% lower than in bass or terrestrial species. Calcium and phosphate levels of both marine classes are higher than in higher vertebrates. The higher alkaline phosphatase level in bass is typical of bony species.

We designed and constructed a system that maintained tank salinity at a 23% reduced level of 26.0 ppt, compared to control seawater of 33.0 ppt during the same 8-week period. This open system consisted of a 4112-gallon specimen tank into which flowed seawater at 8400 ml/min and freshwater from a 33-gallon reservoir at 5000 ml/min. Salinity was monitored daily with an hydrometer. Calcitonin values in sera were obtained with our radioimmunoassay directed against salmon calcitonin and were significantly reduced in animals with chronically reduced calcium levels. Experimental and control bass values were not different, except for hematrocrits.

The two species differ very dramatically in their ability to maintain a constant internal environment independent of the tank environment. The sharks' sera reflected the altered environment without any apparent ill effects. Analysis of vitamin D metabolites and vitamin D hydroxylase activity in kidney homogenates are in progress using HPLC and binding assays, as described in our earlier report. At the beginning of the experiment, control and experimental sharks and bass received subcutaneous implants of demineralized bone powder (DBP) to monitor skeletal formation. Samples harvested at the end of the experimental period are being quantified by histomorphometric evaluation.

We have made the following additional observations: (1) that bass (osteichthyes) in a hyposalinity tank have the ability to resorb implanted bone powder, whereas sharks (chondrichthyes) do not; (2) that calcitonin produces a lowering of blood calcium in bass, whereas it increases blood calcium in sharks; and (3) that the production of calcitonin by sharks is fairly stable despite changes in their environment. We have detected by radioimmunoassay high levels of circulating calcitonin in rays and sharks, the most primitive species in which calcitonin has been found. The administration of salmon calcitonin to sharks produced a prompt increase in serum calcium, in marked contrast to the expected hypocalcemia seen in mammals and produced by us in bass. These data suggest major differences between the biological effects of the hormone on shark and on other mammalian and marine species. These biological differences imply a unique chemical structure for shark calcitonin and warrant its further study.

We thus propose to isolate shark calcitonin from *Triakis semifasciata* and to determine its biological features. These chemical studies will be performed on shark blood and C-cells by extraction, differential precipitation, immunoaffinity chromatography, and HPLC, all of which are established procedures in our laboratories. In a series of comparative studies of bone formation and resorption, we found that marine bass (*Paralabrax*

clathratus) acclimated to hyposalinity develop bone-resorbing cells that are not present in either bass or shark in a marine environment. It is our hypothesis that this hypocalcemic environment causes these fish to produce a parathyroid hormone-like (PTH) factor that recruits the bone-resorbing cells. In support of this view, we have detected circulating PTH-like activity in bass and shark plasma by radioimmunoassay. It is thus likely that PTH-producing cells may be present in fish, a hypothesis contrary to the classic view that discrete parathyroid glands did not evolve until amphibians.

Cooperating Organizations
Ciba-Geigy Pharmaceuticals
Harvard University
Monsanto Corporation
National Institutes of Health
Sandoz Pharmaceuticals
University of British Columbia
University of Goeteborg, Sweden

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Marine Chemistry and Pharmacology Program, Phase II: Metabolites from Marine Invertebrates

Scripps Institution of Oceanography
R/MP-30
Project Initiated: October 1, 1983
Project Completed: September 30, 1986

D. John Faulkner

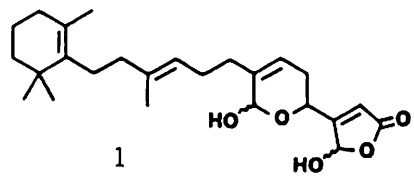
Our research on manoalide (**1**) and its derivatives is almost complete, and all further studies will be performed at Allergan Pharmaceuticals. In January 1985 we collected ~250 lbs (wet weight) of the sponge *Luffariella variabilis* with the intention of isolating sufficient manoalide to complete an initial clinical trial. The unexpected discovery that some specimens contained luffariellin A (**3**) and luffariellin B (**4**) in place of manoalide (**1**) and seco-manoalide (**2**) severely disrupted the project, since every specimen of sponge had to be extracted individually and the extract analyzed by ^{1}H NMR. Of 410 specimens examined in this manner, 22 specimens contained only luffariellins, 32 specimens contained mixtures of manoalides and luffariellins, and only 4 specimens contained neither group of compounds. The structures of luffariellins A (**3**) and B (**4**) have been elucidated by analysis of spectral data. The luffariellins have almost identical pharmacological activity to manoalide and seco-manoalide. Luffariellolide (**5**) was isolated from a second *Luffariella* sp. from Palau (Albizati, et al., in press). Although luffariellolide (**5**) is less active than manoalide (**1**) as a PLA_2 inhibitor, the less complex chemical structure makes it a more appropriate target for chemical synthesis.

In collaboration with Professor C. B. Rao, Andhra University, India, we are studying a new group of alkaloids from a species of *Zoanthus* (Rao et al., 1984; Albizati and Faulkner, 1985). In addition to the three compounds described in the last report, we have now identified two new metabolites (**6,7**) in the same series. These compounds are currently being studied by Professor Jacobs. We hope to continue to study these compounds, but the future of this research depends on finding a way to support the initial isolation work in India.

The burrowing sponge *Siphonodictyon coralliphagum* yielded a series of seven metabolites, exemplified by siphonodictyal B (**8**) and siphonodictyal D (**9**), that inhibit the growth of corals and can assist in the burrowing process by chelating calcium ions (Sullivan, 1985).

Methyl 6-bromoindole-3-acrylate (**10**) was obtained from a marine sponge (Dellar, Djura, and Sargent, 1981). The 6-bromoindole derivative (**10**) inhibits the division of fertilized sea urchin eggs. In order to determine whether the position of bromine substitution was an important factor for biological activity, both methyl 6-bromoindole-3-acrylate (**10**) and methyl 5-bromoindole-3-acrylate (**11**) were synthesized. The 5-bromo derivative (**11**) was devoid of activity in the sea urchin egg assay. However, when assayed against the larvae of the marine worm *Phragmatopoma californica*, the 5-bromo derivative (**11**) was toxic while the 6-bromo derivative (**10**) was not. These results suggest that the desired cytotoxicity of the 6-bromo derivative (**10**) may be due to a very specific mechanism of action.

The γ-hydroxybutenolide ring system found in manoalide (**1**), luffariellolide (**5**) and other marine natural products is thought to be formed by oxidation of the corresponding furan. This reaction can be performed in the laboratory using singlet oxygen. However, the oxidation of a 3-substituted furan (**12**) usually gives a mixture of the required γ-hydroxybutenolide (**13**) and an unwanted isomer (**14**). We have recently developed reaction conditions for the singlet oxygen oxidation reaction that suppresses the formation of the unwanted isomer and has allowed regiospecific generation of the desired γ-hydroxybutenolide (**13**)



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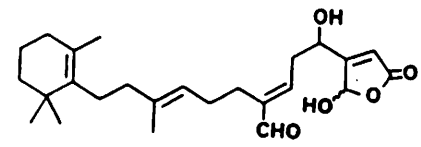

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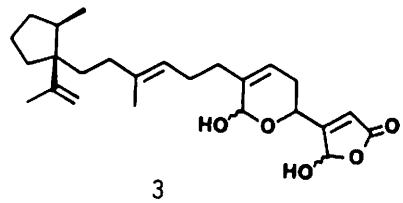

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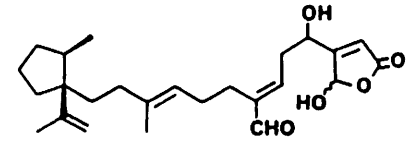

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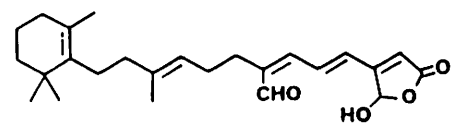

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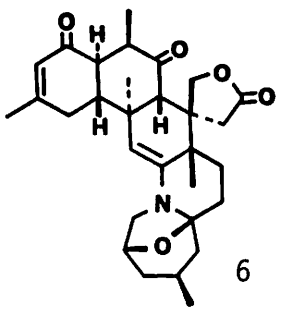

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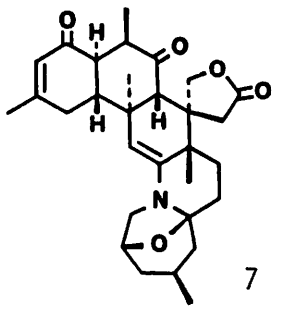

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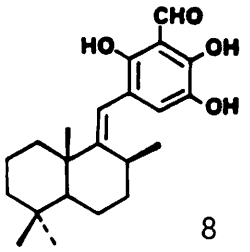

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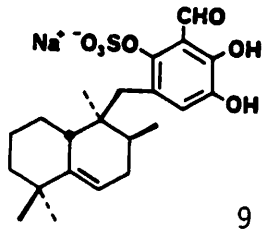

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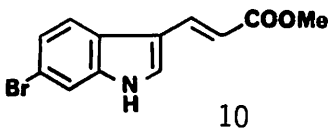

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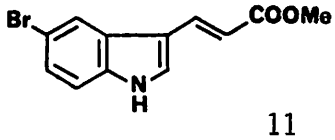
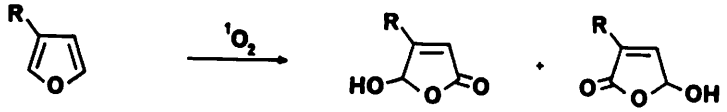


11

sea urchin egg	+	-
worm larvae	-	+



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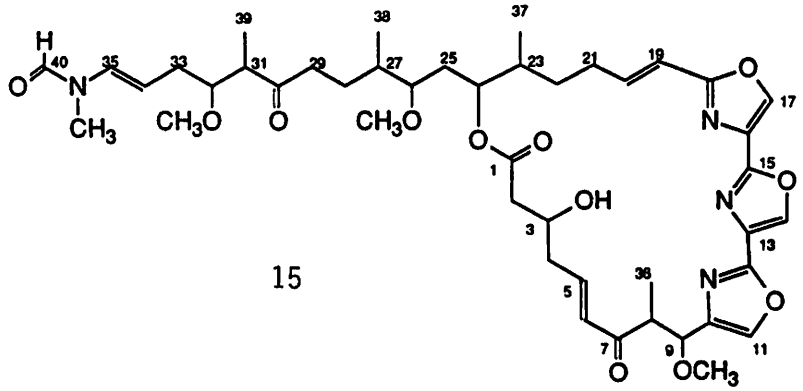

15

from several furans obtained from marine sponges and/or nudibranchs. Some of the synthetic γ-hydroxybutenolides are being screened for anti-inflammatory activity.

We have recently encountered a group of antifungal macrolides from sponges of the genus *Halichondria*. The structure of halichondramide (**15**) has been elucidated by interpretation of spectral data, but the stereochemistry of this complex molecule remains to be determined (Faulkner, 1986). The related metabolites kabiramides B and C were previously found in nudibranch egg masses (Matsunaga et al., 1986). We have found the same compounds in a species of *Halichondria* and propose that the compounds are sponge metabolites that are concentrated by a spongivorous nudibranch and incorporated into the egg masses in order to deter predators.

Cooperating Organizations

Allergan Pharmaceuticals
Smith Kline and French
Syntex

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Lectures

Third International Conference on the Biology of Sponges, Woods Hole, Massachusetts, November 1985.

Phillip Morris Company, Richmond, Virginia, November 1985.

Gordon Conference on Marine Natural Products, Oxnard, California, February 1986.

U.S.-Japan Seminar on Bio-organic Marine Chemistry, Okinawa, Japan, June 1986.

ACS Annual Meeting, Anaheim, California, September 1986.

Conference on Brominated Marine Natural Products, Salford, England, September 1986.

Marine Chemistry and Pharmacology Program, Phase II: Pharmacological Screening and Evaluation

University of California, Santa Barbara
R/MP-31
Project Initiated: October 1, 1983
Project Completed: September 30, 1986

Robert S. Jacobs

The major goals of our project are to (1) test a variety of unique marine natural products for pharmacological activity, and (2) investigate the site and mechanism of action of compounds which may prove to be pharmacologically useful, either clinically or as tools for research.

Seven distinct ring systems are under investigation as analgesic and anti-inflammatory agents in our laboratory. In addition, two potent inhibitors of cell division have been discovered.

Cell Division

Studies with pseudopterosin A were undertaken to investigate the mechanism of action of this compound. Our early findings suggested that this compound blocked mitosis when the chromosomes were condensed, and the nuclear envelope should have depolymerized. What was observed, in fact, suggested that this step in the cell cycle was desynchronized; that is, the nuclear envelope did not break down and microtubules did not form. In contrast to studies with other marine natural products (stypoldione and elatol), microtubule assembly was not affected by pseudopterosin A. In fluorescence studies we were able to positively verify the unusual state of the nucleus following pseudopterosin A treatment. Interestingly this anti-mitotic effect was not common to all analogs of pseudopterosin A. In fact some of the active anti-inflammatory compounds do not seem to inhibit cell division in equivalent concentrations. We have completed the initial studies of the site of action of this compound and submitted it for publication to the the *Journal of Molecular Pharmacology*.

PC 191, a "cyclic peptide," has shown potent inhibitory activity against dividing sea urchin eggs (ED_{50} = 0.1 µg/ml). A patent on this compound has been applied for by Syntex and Dr. Phil Crews. Since this group is working with PC 191 we will not study the compound further. It is interesting to note, however, that the sea urchin assay continues to serve as a useful model for the detection of potent anti-fungal agents.

A new bromoindole compound (JF 284) has been isolated by Dr. John Faulkner. This compound inhibits cell division at concentrations below 0.1 µg/ml and as such represents the most potent compound yet from the screening effort. We will begin to characterize its mechanism of action in coming months.

Anti-Inflammatory Studies

This continues to be one of the most productive areas of investigation. This year we will file patent applications on JF 281, 282, 283, and JF 336. This ring system is new. Although rare and complex, the series remains of great interest to us. These compounds are potent anti-inflammatory agents and analgesics. We have at this time been unable to establish a mechanism for a continuous supply; however, Faulkner is working on this problem.

WF 229 and WF 228 are two new groups of compounds that were isolated by Dr. William Fenical. We have found these compounds to be quite active, and we might pursue them at a later time.

Finally, a group of compounds that are analogues of WF 176 and a prostaglandin analogue, WF 183, have been found to be active anti-inflammatory compounds.

Overall we have now identified 39 active anti-inflammatory compounds representing a variety of new and old ring structures. This last year we focused on manoalide analogues and pseudopterosin A analogues. Patents are pending on manoalide and its analogues. This class of compound has been licensed by Allergan, and formulation and toxicity studies are underway for an eventual clinical trial. Pseudopterosin A patents have been applied for and preliminary studies undertaken at Smith Kline and French laboratories, which might seek an option to license this series this year. Pseudopterosin A has a (CNS) side effect that we have separated from its analgesic effects and anti-inflammatory effects in structure activity studies.

Cooperating Organizations

Allergan
Smith Kline and French Laboratories
Syntex

Publications

De Carvalho, M. S. and R. S. Jacobs. 1986. Suvanine—A novel anticholinesterase from a marine sponge. *Fed. Proc.* 45:581. Abstract.

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Master, M. M. and R. S. Jacobs. 1986. *In vitro* inactivation of hepatic microsomal phospholipase A_2 by the marine natural product manoalide. *Fed. Proc.* 45:581. Abstract.

Marine Chemistry and Pharmacology Program, Phase II: Chemical Studies of Tropical Marine Algae and Coelenterates

University of California, San Diego
R/MP-32
Project Initiated: October 1, 1983
Project Completed: September 30, 1986

William H. Fenical

This project is a component of a tripartite collaboration between scientists at the Scripps Institution of Oceanography and the University of California, Santa Barbara, called the Marine Chemistry and Pharmacology Program. The long-term objectives of this program are threefold: (1) to generate baseline chemical and biological data that define the chemical and physiological activities of tropical benthic marine plants and animals, (2) to isolate and define novel organic compounds from marine organisms which are of use in defining and elaborating biochemical mechanisms and pathways involved in human disease, and (3) to apply these latter findings toward the development of novel classes of new drugs.

The research proposed for the 1983–86 period was described as a "Phase II" effort. This designation was in response to the fact that a significant number of discoveries had been made in prior years, but not developed. Thus, a major goal of the last three years was to progress on the developmental front, from both the University of California legal perspective and the industrial development point of view.

Thus, the last three years emphasized the pursuit of existing leads toward patenting and the introduction of those leads to our industrial collaborators. What follows is a brief comprehensive description of our activities for the last three years.

Field Programs

Although at a less aggressive pace, we have continued to explore marine organisms for their novel secondary metabolites. This exploration was accomplished by two separate mechanisms, one involving our use of the University of Miami's research vessels, ORV *Calanus, Cape Florida*, and *Columbus Iselin* in the late summers of 1984, 1985, and 1986. These expeditions brought us ample opportunity to collect and document numerous marine plants and animals from the Caribbean Sea. Additional field programs also were established in Baja California, Sea of Cortez, and in the Indo-Pacific regions of South China and the Philippines.

In all these efforts, marine species which produce new compounds possessing some form of biological activity were identified. Specimens were returned to La Jolla, and they have been chemically investigated. While not all these investigations can be mentioned in detail, some are illustrated below.

Chemical Studies

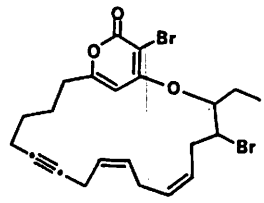
Chemical studies have stressed marine algae and soft corals because of our long term interest in these groups of organisms. For example, we have isolated several antimicrobial cyclic macropyrones (**1-4**) from the Indopacific red alga *Phacelocarpus labillardieri*. Other compounds from marine algae consist of the new chamigrene derivatives (**5-6**) isolated from the Chinese alga *Laurencia guandongensis*. These latter compounds show activity in several areas including insecticidal, herbicidal, and antibacterial activities.

More extensive research has been conducted with gorgonian and telestacean corals from several habitats. The Chinese gorgonian *Subergorgia suberosa* was found to contain the unusual tricyclic sesquiterpenoid **7**, which has potent cardiac toxicity in test systems. While toxins do not normally generate new drugs, they have proven essential in basic biomedical research, acting as molecular probes to define the biochemistry of disease. From a related gorgonian coral from Palau, *Solenopodium* sp., we have isolated a new group of briarein diterpenoids possessing antiviral activities. The new compounds, the solenolides (**8-10**), are closely related to the well-known diterpenoid briarein A. The potent antiviral activity of this group, however, had not been recognized.

A telestacean coral from the Indopacific (Aldabra Atoll), identified as *Coelogorgia palmata*, was found to produce three related sesquiterpenoids (**11-13**), including a new metabolite of the "bakkane" class (**13**). These metabolites are currently in comprehensive biotesting.

What is perhaps our most exciting discovery is the rather widespread anti-inflammatory activity found in Caribbean gorgonian corals of the genus *Pseudopterogorgia*. *P. kallos*, for example, was found to produce a series of "pseudopterane" diterpenoids called the kallolides, and exemplified by structure **14**. Kallolide A, for example, is a potent anti-inflammatory agent competitive with the drug indomethacin in its overall effects. Several other compounds isolated from this source cannot be mentioned here due to premature disclosure problems.

By far the most important discovery has been the "pseudopterosins," a new class of compounds isolated from various *Pseudopterogorgia* species, e.g., *P. elisabethae*. This discovery represents a new class of over 20 natural products and synthetic derivatives possessing potent anti-inflammatory and analgesic



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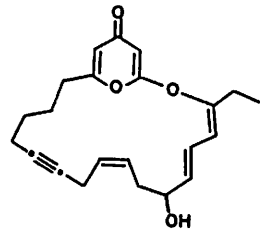

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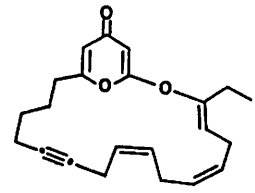

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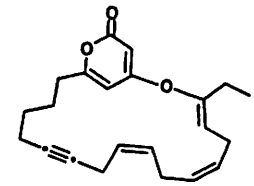

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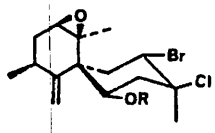

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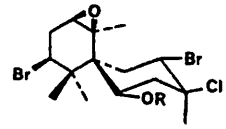

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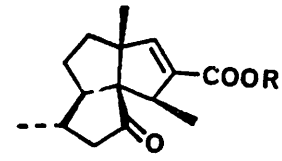

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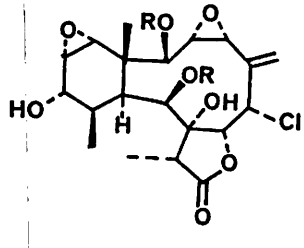

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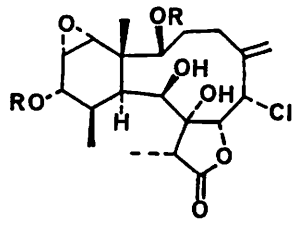

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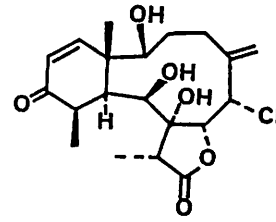

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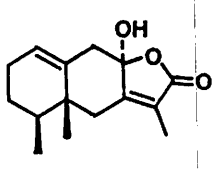

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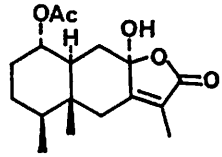

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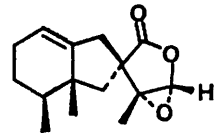

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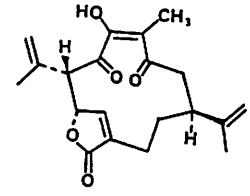

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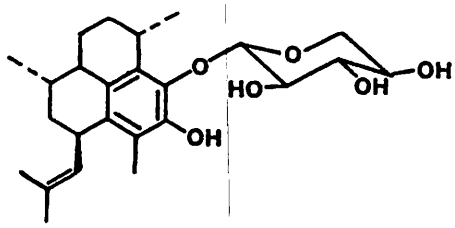

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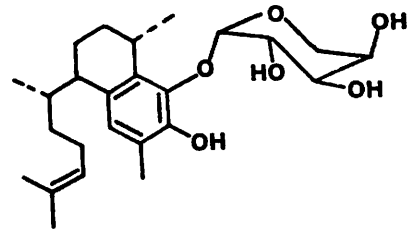

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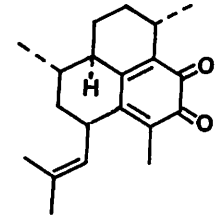

17

properties. The pseudopterosins (as in **15**) and the seco-pseudopterosins (as in **16**) represent the major naturally occurring compounds in this series. Synthetic derivatives have been prepared via operating upon the side chain to produce saturated and oxygenated derivatives. In each case, the derivatives produced maintained the potency of the natural products, and in some instances the derivatives reduced unwanted but minor side effects.

It is of considerable importance to identify the mechanism of action of these new compounds. Once identified, a clearer picture can evolve as to the therapeutic applications of the pseudopterosins. It has been demonstrated, however, that the pseudopterosins are not acting as standard anti-inflammatory drugs, i.e., as inhibitors of cyclooxygenase. There appears to be a new mechanism of action involved here, perhaps one involving the inhibition of phospholipase. To probe the details of the mechanism, we have been working closely with Professor Jacobs to synthesize possible intermediates and to test possible breakdown products. We have synthesized the quinone **17**, since it is entirely possible that this compound is liberated *in vivo* as part of the metabolism of the pseudopterosins.

Patenting and Commercial Development

Although we have submitted 9 disclosures of possibly patentable discoveries to the University of California Patent Office, only one case has received favorable attention. Patents are now pending on the pseudopterosins in both the United States and the international marketplace. The patent application is generic in substance, in that it claims numerous derivatives to be active. On the basis of substantive experimentation, we feel that the intact glycoside linkage is absolutely imperative to the biological activity of the pseudopterosins.

There has been considerable interest expressed by the pharmaceutical industry concerning the pseudopterosins. We have provided several industries, such as Allergan Pharmaceuticals and Smith, Kline and French Laboratories, with significant quantities of these compounds as a preliminary gesture to secure their interest. Negotiations are in progress to secure an option agreement for the exclusive patent protection for these compounds during development.

Cooperating Organizations

Allergan Pharmaceuticals, Irvine
Smith Kline French Laboratories, Philadelphia, Pennsylvania
Syntex Research Laboratories, Palo Alto

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Lectures and Conferences

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Fenical, W. H. 1985. Marine Biotechnology. Presented at University of Maryland, School of Pharmacy, October 12, 1985.

Fenical, W. H. 1985. Bioactive metabolites from marine sea fans and whips. Plenary Lecture at 16th Annual Meeting of the Chilean Chemical Society, December 5, 1985.

Fenical, W. H. 1986. New leads for anti-inflammatory drugs. Presented at McNeil Laboratories, March 10, 1986, Philadelphia, Pennsylvania.

Fenical, W. H. 1986. Marine natural products as exploratory leads in the development of anti-inflammatory drugs. Presented at University of California, Los Angeles, Chemistry Department, June 12, 1986, Los Angeles, California.

Marine Chemistry and Pharmacology Program, Phase II: Natural Products from Toxic Marine Organisms

University of California, Santa Cruz
R/MP-33
Project Initiated: October 1, 1983
Project Completed: September 30, 1986

Phillip Crews

The natural products chemistry of terrestrial plants or animals has long provided important leads in pharmaceutical research. In looking to the future, marine research offers the alternative of providing new biomedicinal leads. This possibility was recently emphasized in statements made by Dr. David Attaway (National Sea Grant College program): "The diminishing returns from continued screening of terrestrial organisms for new drugs, the slow development of methods for making synthetic substances with specific pharmacological properties, the lack of effective treatment for many diseases, and the continuing development of resistance to drugs by pathogenic organisms mandate the exploitation of marine biochemicals." Recently, other detailed arguments in support of this same thrust appeared in the report "Our Nation and the Sea," issued by the Presidential Commission on Marine Science, Engineering and Resources (January 1979) (Hashimoto, 1979) and in articles tracing the development of marine natural products (Naylor, 1984, Riegle, 1982).

Recent research has clearly shown that chemistry of marine organisms is quite novel (Crews and Naylor, 1985). Alongside this are interesting pharmacological observations, such as that extracts from marine organisms have bioactivity in a variety of assays (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 thrust during the past three years has been to continue development of novel marine animal natural products with promise in several relevant pharmacological systems. Last year our collaborators and general areas of investigation were:

(1) Dr. Tom Matthews, Institute of Antimicrobial & Antiviral Chemotherapy, Syntex Research—Compounds of interest exhibit activity in the areas of antifungal and antiparasite.

(2) Dr. Jake Clemens, SeaPharm—Compounds of interest exhibit activity in the areas of antitumor and immunomodulation.

(3) Dr. Mark Schroeder, Shell Development Labs—Compounds of interest exhibit activity in anti-insect assays.

(4) Prof. Robert Jacobs, University of California, Santa Barbara, Sea Grant Project—Compounds of interest exhibit activity in the areas of skeletal muscle, anti-inflammation and cell division inhibition.

Bioassay-guided isolation work on crude extracts continued to be important and was supported by our ties with industrial collaborators. It incorporated the rapid two-way communication of results (for an example of this, see Crews et al., 1984) and shuttling of samples between laboratories. Bioassay isolation and purification of active constituents from crude extracts were carried out at University of California, Santa Cruz in our well-equipped marine natural products laboratory. Structural elucidations were performed on purified compounds, utilizing state-of-the-art equipment and approaches. We often utilized NMR techniques and strategies that we have pioneered. (Crews et al., 1985; Crews and Bescansa, 1986). Some highlights of recent results are summarized here.

Our collaborative work with Syntex was stimulated by the promising results obtained with crude extracts (Table 1) and pure compounds (Table 2). All of these extracts and compounds were obtained from materials collected during expeditions to Tonga and Fiji. Two years ago we isolated and characterized (S)-l-tridecoxy-2,3-propanediol, (Table 2, PC-98) (Myers and Crews, 1983) from an undescribed Tongan sponge, and this compound showed positive results in an *in vitro* anthelmintic assay. Our Syntex collaborators requested two grams of synthetic compounds (Table 2, PC-99, and PC-100) for *in vivo* assay. We responded with synthetic, optically pure material which was moderately active against a mixed helminth infection in mice. A similar study, which has produced more significant results, involves our discovery of *Jasplakinolide* (1) from extracts of a Fiji sponge, *Jasplakina* sp. The crude extracts of our first collection of less than a kilogram of this sponge (coll. # 84-29, Table 1) showed significant activity in anti-fungal assays versus *Candida albicans* (crude extract @100 μg/ml = 33 mm inhibition of growth zone). Re-collection of 5.5 kg (coll. # 85-5 and 86-3) of this sponge yielded, by bioassay-guided isolation, jasplakinolide (I). Its complete structure, but without stereochemistry was elucidated and disclosed in a U.S. patent application (USSN 824,056). A summary of its pharmacological properties appears in Table 3. The antifungal data for I is impressive (mic = mlc = 25 μg/ml) is higher than

Table 1. Samples with *In Vitro* Antifungal and Anthelmintic Promise

	Bacteria			Fungi			Proto.	Myco.	Worm	Virus			Cell Tox (µg/ml)		
#	EC	PA	SA	SP	CA	TM	TC	MY	NB	H1	H2	H3	MRC	HEP	RMK
A. Selected Crude Extracts, Tonga Expedition															
841							-/+						10	10	
843							-/+						100	100	
844			+	+	+	+	-/+	+	+				10	10	
846			+		+	+	-/+						10	10	
847									+					3.2	
8414							-/+						10	1	1
B. Selected Crude Extracts, Fiji Expedition															
8415			+	+			-/+						10	.32	.32
8418							-/+						10	3.2	3.2
8419									+					1	1
8422			+	+			-/+						10	10	10
8425			+	+			-/+						10	32	32
8528			+	+			-/+		+				1	1	1
8429					+				+				.01	.32	.32
8430				+	+	+	+/		+				.0001	.32	1

A blank space indicates no activity. Organisms are as follows: *E. Coli; Psuedomonas aeruginosa; S. aureus; Streptococcus pyrogenes; C. albacans; T. mentagrophytes; Trypanosoma cruzi; Mycoplasma* sp.; *Nippostrongylus braziliensis*; H1 = HSV_1; H2 = HSV_2; H3 = parainfluenza V_3; HEP, human epithelial carcinoma of larynx; MRC, human embryonic lung, diploid; RMK, Rhesis monkey kidney cells

the commercial pharmaceutical clotrimazole whose mic = 100 mcg/ml and mlc > 200 mcg/ml), and *in vivo* assay demonstrates that this compound is as active as clotrimazole. Commercial fungicides such as clotrimazole typically contain imidazole functionality, whereas the structure of jasplakinolide (I) is quite different; consequently, it represents a new concept of a molecular structure possessing this type of activity. A similarly productive project involved bioassay-guided isolation of cyclic peptides from the sponge 8430 by massive re-collections of it (85-9, 86-9). The structures of two compounds, bengamide A (2) and bengamide B (3), have been established and a U.S. patent application (USSN 875,486) has been filed. The pharmacological properties of these compounds are summarized in Table 4.

A biological testing agreement was completed between ourselves and SeaPharm. This relatively new company is developing new pharmaceuticals from marine sources, and we were able to obtain assay data in several new categories. The most important were in the area of anti-tumor activity, which are summarized in Table 5. Compounds with cyclic peroxide functionality have been especially active as *in vitro* cytotoxins. This has included nuapapuic acid, (Manes, Bakus, and Crews, 1984) (PC-167) and muqubilin (PC-103) from a Tongan sponge, *Prianos* sp. Two other examples with significant activity are the xestins from a Fiji *Xestospongia*

Table 2. Compounds with *In Vitro* Antifungal and Anthelmintic Activity

#	Name	Bacteria				Fungi		Proto.	Myco.	Worm
		EC	PA	SA	SP	CA	TM	TC	MY	NB
PC-14	Chondrocole A				+	+	+	+	+	—TF—
PC-42	Aerothinin-I	+		+	+			+	+	
PC-52	Heteronemin				+			+		
PC-98	Ether Glyceride				+		+	+	+	
PC-99	(-R) Ether Glyceride				+			+	+	
PC-100	(+S) Ether Glyceride			+	+		+	+	+	
PC-102	Polybromo Phenol Ether			+	+		+	+	+	
PC-103	Muqubilin Acid				+			+		
PC-132	Heteroneminfuran			+	+		+	+	+	
PC-169	12-Episcalaradial			+	+			+	+	
PC-170	12-Episcalarin							+	+	
PC-171	12-Deacetyl-12-Episcalaradial			+	+		+	+	+	
PC-172	12-Acetyl-24,25-Dimethyloxyscalarin			+	+			+	+	

Table 3. Jasplakinolide (1), from Sponge 84-29: Antifungal and Anthelmintic Activities

A. *In Vitro* Bacterial - Fungal @ 100 µg/ 13 mm disk:

Organism	Zone Size
E. Coli	na
Psuedomonas aeruginosa	na
S. aureus	na
Streptococcus pyrogenes	na
C. albacans	20 mm :mic = mlc = 25 µg/ml (broth)
T. mentagrophytes	na
Mycoplasma sp.	na

B. *In Vitro* Nematode (*Nippostrongylus brasiliensis*) @ 3 µg/ml:

casts	0%
motility	0%
viablilty	0%

comment: complete activty at this concentration
ED_{50} < 1 µg/ml

C. *In Vitro* Viral

Organism	Viral Rating
HSV1 F	0.05
HSV2 G	0.05
HSV2 lovelace	na
ParaInfluen 3 C243	na
RSV long	0.32

comment: minimum viral rating of 0.5 = marginal activity

D. *In Vitro* Cell Toxicity (µg/ml)

Organism	Partial	Complete
HEP (epithelial carcinoma, larynx)	0.32	1.0
MRC (human embryonic lung, diploid)	0.01	–
RMK, Rhesis monkey kidney cells	0.32	1.0

E. *In Vivo* Antifungal method: topical treatment of a mouse vaginal *C. albicans* infection. A four day treatment, once daily.

		Percent Negative		
Treatment	Con.	Day 4 post infect	Day 8 post infect	Result
Jasplakinolide (1)	2%	53	47	active
Miconazole nitrate	2%	47	47	active
Placebo	0%	07	07	not active
Untreated	–	07	07	not active

sp., which exhibited *in vitro* antitumor activity against P388 and L1210 test systems, and these data are summarized in Table 6. Unfortunately, none of these compounds has demonstrated *in vivo* anti-tumor activity. Another parallel project involved sponge # 8502 which contains a series of isomalabaricane triterpenes. This project has not been completed but data obtained to date are summarized in Table 7.

We concluded a three-year joint research project with the Biological Sciences research group at Shell Development. Our collaboration was with Dr. M. Schroeder. This project involved compounds of the briarein-A class, which are diterpenes derived from soft corals. Initial assays on a crystalline sample thought to be briarein B (7) exhibited potent corn ear worm anti-feedant activity and aphid toxicity. Briarein-B had been encountered by others but the regiochemistry of the five ester substituents has never been established. We began to purify our active sample of briarein-B but found it to be a mixture of four compounds. The structures of each of these has been established, (Eid and Crews, 1985) including ester regiochemistry, by employing 2D COSY long-range experiments. Purified samples of each were submitted for retesting along with samples of several additional metabolites, and a complete summary of these results is shown in Table 8.

Our collaboration with the Jacobs Sea Grant-supported project was also productive. The most important project involved continuing developmental work on a Fiji sponge sesterterpene, suvanine (**12**) (Manes et al., 1985), isolated from *Ircinia* sp. Suvanine was shown to be a potent acetyl choline esterase (ACH) inhibitor that operates at the molecular level, and the exact mechanism of action was extensively probed by the Jacobs group. Prompted by continuing requests for more compound and derivatives, we were recently able to re-collect the organism that is the source of this compound. An additional supply of this compound along with new stereochemical insights derived by intensive NMR study have prompted us to propose a revised structure, which will be the subject of a future publication. Another project with Jacobs concerned the isolation and evaluation of scalarane-type sesterterpenes (for a recent review of sesterterpene chemistry see Crews and Naylor, 1985) in anti-inflammatory assays. Included in this study were a series of 10 substituted scalaranes from the sponge *Hyrtios erecta*, and the sponge *Carteriospongia* sp. Jacobs has also found that Jasplakinolide is very potent in his anti-inflammatory and anticell division assays.

Cooperating Organizations

SeaPharm
Shell Development
Syntex
University of California Research Expeditions Program

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Table 4. Bengamides, From Sponge 84-3O: Antifungal and Anthelmintic Activities

Bengamide A (2)

A. *In Vitro* Bacterial - Fungal Broth

Organism	Result
E. Coli	na
Psuedomonas aeruginosa	na
S. aureus	na
Streptococcus pyrogenes	mic = 3.9 µg/ml
C. albacans	na
T. mentagrophytes	na
Mycoplasma sp.	na

B. *In Vitro* Nematode (*Nippostrongylus brasiliensis*) @ 50 µg/ml:

casts	5%
motility	0%
viability	0%

comment: complete activity at this concentration

Bengamide B (3)

A. *In Vitro* Bacterial - Fungal Broth

Organism	Result
E. Coli	na
Psuedomonas aeruginosa	na
S. aureus	na
Streptococcus pyrogenes	mic = l.9 µg/ml
C. albacans	na
T. mentagrophytes	na
Mycoplasma sp.	na

B. *In Vitro* Nematode (*Nippostrongylus brasiliensis*) @ 50 µg/ml:

casts	5%
motility	0%
viability	0%

comment: complete activity at this concentration

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Table 5. Extracts With *In vitro* Antitumor Activity[1]

Tumor[2]	P388			Lung			Mammary			Colon		
@µg/ml[3]	50	5	0.5	50	10	1	50	10	1	50	10	1
Sample												
80-1C	4	1										
8401	4	1										
8404	4	2										
8405	3	1										
8406	4	3										
8411	4	4										
8415	4	3										
8417	4	4										
8425	4	2										
8426	4	3										
8432	2	2										
8502	4	3	2	4	4	3	4	4	2	4	4	3
8506	3	2	na	4	3	1	4	4	2	4	3	na
8509	4	3	1	4	3	2	4	3	2	4	3	3
8513	2	na	na	4	2	1	4	1	na	2	na	na
8514	3	3	2	4	4	3	3	3	2	4	3	3
8515	4	3	na	4	3	1	4	2	1	4	3	2
8516	4	1	na	4	3	1	3	2	na	4	2	na
8517	4	1	na	4	4	1	4	4	na	4	4	na
8524	4	4	na	4	4	na	4	4	1	4	4	2
8525	4	3	na	4	3	2	4	4	3	4	4	3
8532	2	na	na	2	1	1	3	na	na	3	na	na
8538	3	1	na	4	1	na	4	1	1	3	na	na
8540	4	3	2	4	4	4	4	4	3	4	3	2
8541	3	na	na	4	1	1	4	na	na	4	na	na
8552	3	2	1	4	4	2	4	3	2	4	3	2
8553	4	3	1	4	4	1	4	4	1	4	3	1
8561	3	na	na	4	na	na	3	1	1			
8562	4	4	na	4	4	na	4	4	1	4	4	1
8566	4	3	na	4	3	1	4	3	2	4	4	na
8568	3	3	na	4	2	1	4	3	na	4	2	na

NOT TESTED

[1]The activities are coded as follows: na, no activity; 1, 75-89% of cells still viable; 2, 50-75% of cells still viable; 3, 25-49% of cells still viab1e; 4, less than 25% of cells still viable.

[2]Tumors are as follows: P388, standard murine leukemia cells; lung, A-549 cells; Mammary, MDAM8231 cells; colon, HCT-8 cells.

[3]A total of 72 samples were tested and 31 were active in one or more categories.

Table 6. Summary of Sponge # 8417 (*Xestospongia* sp.) *In Vitro* Antitumor Activity[1]

Tumor[2]	P388				Lung			Mammary			Colon		
@mcg/ml	50	5	0.5	IC_{50}	5	0.5	0.05	5	0.5	0.05	5	0.5	0.05
crude extract	4	4											
Butenolide (4)	2	na	na										
Xestin A (S)	4	4	4	0.3 µg/ml	4	na	na	4	na	na	4	na	na
Xestin B (5)	4	4	na	3.0 µg/ml	na	na	na	na	na	na	na	na	n

[1]The activities are coded as follows: na, no activity; 1, 75-89% of cells still viable; 2, 50-75% of cells still viable; 3, 25-49% of cells still viable; 4, less than 25% of cells still viable.

[2]Tumors are as follows: P388, standard murine leukemia cells; lung, A-549 cells; Mammary, MDAM8231 cells; colon, HCT-8 cells.

Table 7. Summary of Sponge # 8502 (*Jaspis stellifera*) *in vitro* Antitumor Activity[1]

Tumor[2]	P388			IC_{50}	Lung			Mammary			Colon		
@ µg/ml	50	5	0.5		5	0.5	0.1	5	0.5	0.1	5	0.5	0.1
Sample													
85-2α Fract A	4	4	2	0.6	4	3	1	4	1	ND	3	ND	ND
85-2α Fract B	4	4	4	0.1	4	4 0.05 µg=2 0.01µg=ND	3	4	3	ND	4	2	ND
85-2α Fract C	4	4	1-2	3.0	4	4 0.05 µg=3 0.01µg=2	3	4	3 0.05 µg=2 0.01 µg=1	4	3	3 0.05 µg=ND 0.01 µg=ND	1

[1]The activities are coded as follows: na, no activity; 1, 75-89% of cells still viable; 2, 50-75% of cells still viable; 3, 25-49% of cells still viable; 4, less than 25% of cells still viable.

[2]Tumors are as follows: P388, standard murine leukemia cells; lung, A-549 cells; Mammary, MDAM8231 cells; colon, HCT-8 cells.

Table 8. Summary of *Briarein* Metabolites with Activity in Agricultural Assays

A. Toxicity (@ 0.1% spray)

Compound	Pea Aphid Activity Level	Corn Ear Worm Activity Level
briarein A (6)	100%	100%
epoxy briarein A (7)	100%	100%

B. Systemic Toxicity (@ 0.5% transport via bean root ball to leaves)

Compound	Pea Aphid Activity Level
briarein A (6)	100%
epoxy briarein A (7)	100%
briarein B (8)	100%
epoxy briarein B (9)	100%
briarein C (IO)	NA
brianthein Z (II)	LC_{50} = 0.28
azodrin (standard)	LC_{50} = 0.36

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Ocean Technology

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The Geology and Structure of the Southern Hosgri Fault Zone Offshore California: A 3-D View Using Computer Color Graphics

Bruce P. Luyendyk

University of California, Santa Barbara
R/OT-10
Project Initiated: October 1, 1983
Project Completed: March 31, 1986

Our goal has been to describe the structure of the southern terminus of the Hosgri Fault Zone offshore south central California, and to interpret this structure in terms of geologic history and geologic hazards. The Hosgri Zone is a major north-trending dextral strike slip fault about which little is known. It may be part of a system on which the third largest earthquake in California occurred in 1927.

The study area ranges from Point Purisima west of Santa Maria to south of Point Arguello, and from the coastline to approximately 10 km offshore (Figure 1). Prior to our work the Hosgri was poorly mapped here both because of the paucity and low quality of past data and because of structural complexities of the area. Extensive offshore oil development also is taking place in the study area.

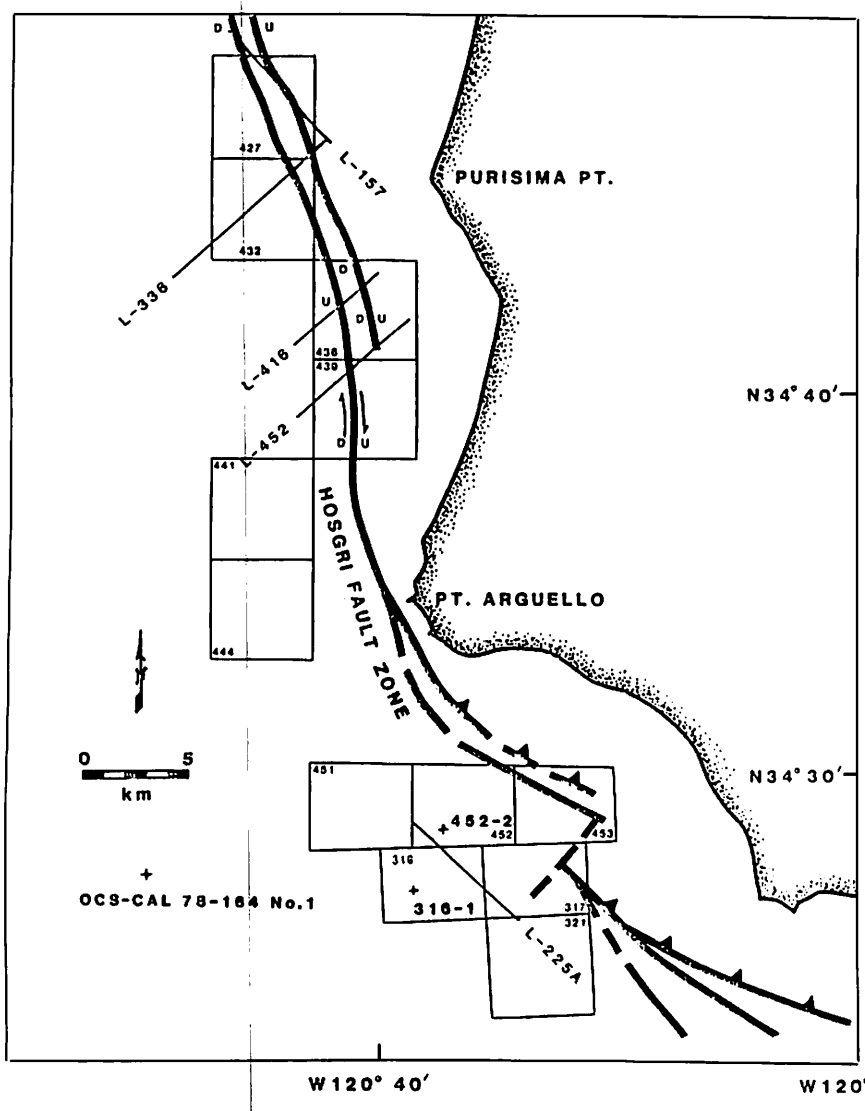

Figure 1. Location map showing federal lease blocks and the location of key seismic profiles and wells. The trace of the Hosgri Fault Zone shown here was mapped during this project.

We had two objectives in mapping this region. One was to understand the apparent joining of the east–west faults in the western Transverse Ranges, such as the Santa Ynez River Fault (or Lompoc-Solvang Fault), with the Hosgri. Another was to find the southernmost terminus of the Hosgri Fault Zone.

We used new seismic data sets to map the fault zone in our study area. These data are high-resolution seismic reflection records obtained for geohazards evaluation from surveys conducted by both the U.S. Geological Survey's Minerals Management Service and by oil companies who hold leases offshore (Figure 2, Table 1). In addition to using traditional interpretation and mapping techniques, we developed a software system to construct computer graphics, 3-D geologic models of the structure beneath the seafloor.

Our interpretation and mapping effort has shown that the Transverse Ranges faults, such as the Santa Ynez River Fault, do not extend a significant distance offshore—at least in the section above 1.0 seconds (round trip) subbottom (Figure 3). The Hosgri Fault Zone is expressed as a single fault trending nearly north–south about 5 km offshore and can be traced south of Point Arguello (Figure 3). Our mapping has shown two facts; first, that the Hosgri Fault Zone extends well south of the Santa Maria Basin

CONTRACTOR	TWO-WAY TIME OF RECORD (seconds)	SOURCE	YEAR RECORDED	FOLD	GENERAL AREA OR FEDERAL LEASE BLOCK NUMBERS	ANALOG DISPLAY	PROCESSED RECORDS		
							MIGRATED	AGC	TRUE AMPLITUDE
Fairfield Industries	1.0	FAIRFLEX mini-sleeve exploder, 6 pairs of sleeves	1979	12	offshore Santa Maria Basin			√	√
				1	451,452,453	√			
Inter Sea Research Corporation	1.5	sparker, 9 tip, 13.5 kj	1981	12	3 mile limit to coastline, Pt. Arguello to Pt. Conception		√	√	√
					317			√	√
Applied Research Concepts	2.5	3 waterguns, 2000 psi	1981	24	427,432 436,439		√	√	√
Nekton	2.0	3 waterguns, 2000 psi	1981	24	441			√	√
Interstate Electronics	2.0	sparker, 9 tip, 8.5 kj	1981	12	444			√	√

Table 1. Summary of seismic reflection data.

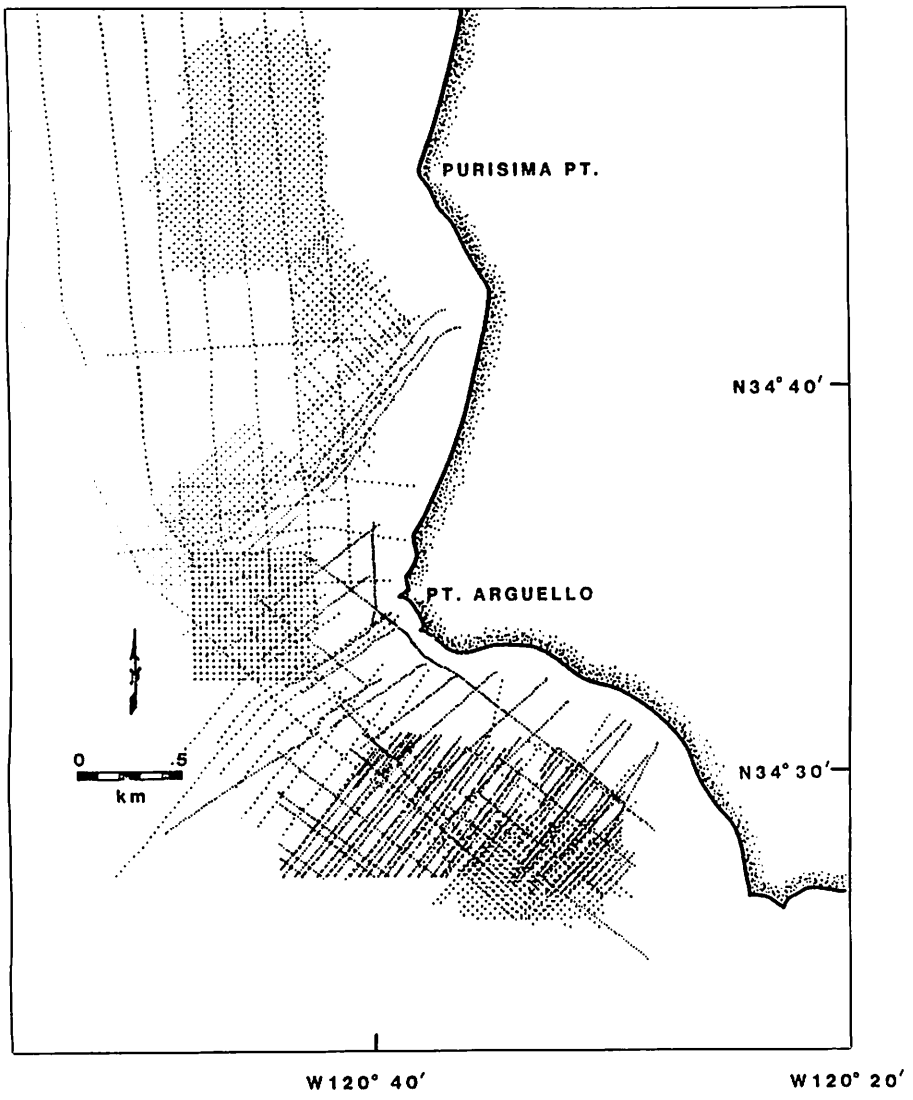

Figure 2. Track line map showing the location of navigation points for the seismic data studied in this project (see Table 1).

and in fact almost reaches Point Conception, and second, that the Transverse Ranges faults are truncated by the zone and do not extend beyond it.

The graphics modeling effort was centered around the graphics software package DI 3000, written by Precision Visuals Inc., and a Tektronix 4113 color graphics terminal. To use the DI 3000 software for our modeling we have written several rather complex programs to adapt our data base for visual display. One major obstacle was that the DI 3000 system is written to graph surfaces that are fully continuous in the area of interest; this is an inappropriate constraint for imaging discontinuous geologic surfaces such as faults and individual horizons. We designed a masking procedure to specify the limits of the horizons we are mapping that is consistent with the requirements of DI 3000. Additionally, we had to overcome the problem of hidden surfaces, that is, causing the surfaces to be drawn so that the ones closest to the viewing point overlay surfaces farther away or deeper. This was accomplished by breaking the surface and fault data into separate files which can be drawn in arbitrary order depending on their position with respect to the viewer; the first-drawn surfaces are overlain by the last in the model. The faults themselves are drawn in sections

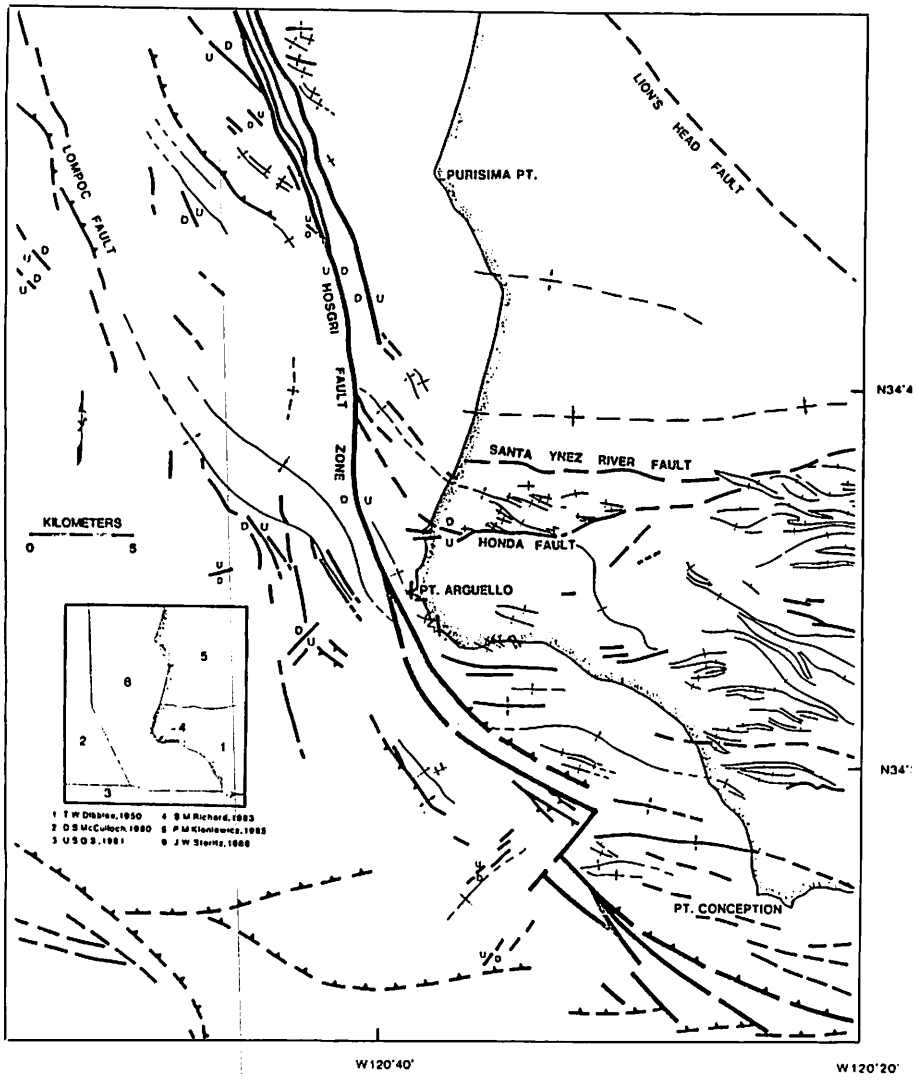

Figure 3. Detailed subbottom structure map produced by interpreting the data base shown in Figure 2 and Table 1.

delimited by the intersections between the faults and the surfaces.

We are able to produce two different types of geologic models. We start by interpreting the seismic record sections on a digitizing table; we digitize three horizons (the seafloor, Foxen/Sisquoc unconformity, and top of the Monterey Formation) and the various faults on the individual sections. These data are then merged with a navigation file so that we have the horizons and faults registered in geographic coordinates. From this data base we can produce the first model type, which is a collection of cross sections or line drawings that we refer to as the "fence diagram." The fence diagram is a 3-D view of all the digitized record sections; each surface is depicted in a different color with the faults in red. The second type of model is a 3-D view showing meshed surfaces (Figure 4). From the original digital data we compute a mask and grid the data to form the individual surfaces; the faults are shown as lines in the plane of the original cross sections. Each surface individually has a hidden line property to facilitate viewing of complex structure.

We experimented with adding flexibility to the viewing parameters for the 3-D models. Our objectives were to facilitate the viewing of the models from various distances, azimuths, and elevations. This is implemented now, as is the ability to view individual faults and surfaces separately.

Cooperating Organizations

California State Lands Commission
Crouch, Bachman and Associates, Inc.
Ogle Petroleum Inc.
U.S. Geological Survey
U.S. Minerals Management Service

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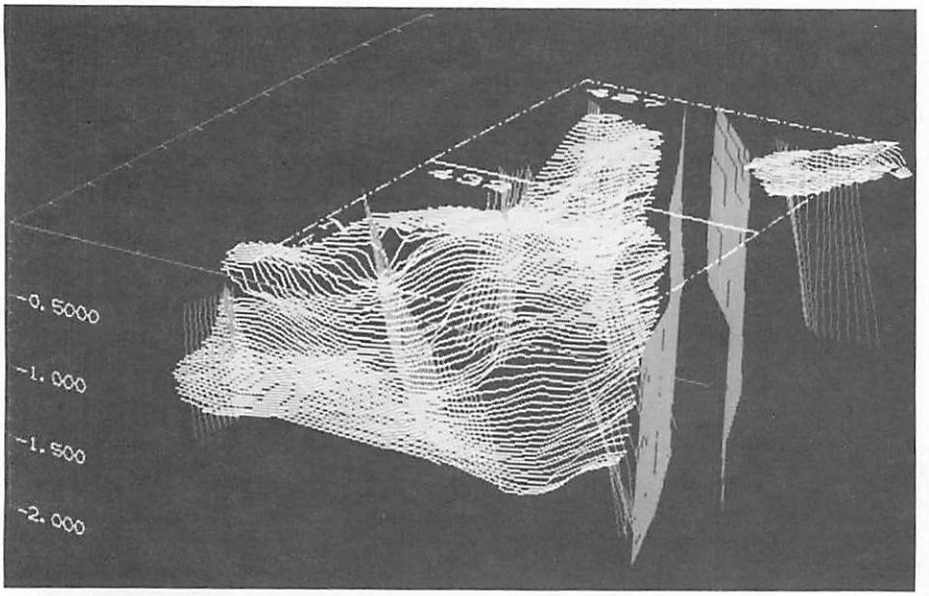

Figure 4. Lease blocks 427 and 432 viewed from S25E looking toward N25W at an inclination of 20 from horizontal. For location of lease blocks, see Figure 1. Fault planes are vertical; an upper Miocene unconformity is horizontal. The vertical exaggeration is about 3 assuming a velocity of 5000 ft/sec in the subbottom sediments. Horizontal axes have ticks at one kilometer intervals; the vertical scale is in 2-way reflection time.

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Development of a Methodology for the Design of an Offshore Oil Production Platform on the Alaskan Arctic Ocean Continental Shelf

University of California, Berkeley
R/OT-11
Project Initiated: October 1, 1983
Project Completed: September 30, 1985

Ben C. Gerwick, Jr.

Design Logic

As a first step in design methodology for production platforms, a design logic has been prepared. The logic has been presented in the form of a logic diagram. The purpose of this logic diagram is to provide a step-by-step design process for structures in the ice environments and to outline the techniques for evaluating the design ice forces. Separate design logic diagrams have been developed for vertical-sided and sloping-sided (conical) structures. The design logic diagram lists the various types of ice features to be considered and provides a sequential listing of analysis techniques.

Ice Mechanics

Since the production structures for the Southern Beaufort Sea will have ice loading as the critical design criteria, it is essential for the designer to be aware of ice as a material. This section provides an overview of the physical and engineering properties of ice. A detailed description of the terminology and definitions relevant to ice mechanics have been presented in this section of the study, along with a complete set of published data for engineering properties, such as compressive strength, flexure strength, shear strength, friction coefficient, etc. With the background of ice mechanics developed through this section, the designer can proceed to the next section for determining the ice forces on the structures.

Ice Force Analysis Techniques

The design of a production platform in ice-infested waters of the Arctic Ocean requires that the structure be able to withstand interaction with ice features such as summer ice floes, multiyear ridges, etc. This section of the design feature is done on a risk assessment basis by ensuring that the probability of encountering the design ice feature during the life of the structure is within acceptable limits.

After the design features have been selected, the next step is to compute the ice forces exerted by these features on the structures. The process of determining ice forces involves evaluation of failure force for various possible failure modes and then selecting the appropriate one as the governing force. This type of analysis is required for each type of feature. This section of the study outlines methods for computing the forces for different types of ice features, for both vertical-sided and sloping-sided structures. The analysis for ice forces on sloping-sided structures involves rather inconvenient equations; therefore, these equations have been solved and generalized solutions presented in the form of nomograms, which are easy to use.

For summer ice floe interaction, state-of-the-art analysis techniques have been updated by introducing an empirical contact factor in the analysis, which improves the validity of computation of the interaction force.

Local Ice Pressures

The above-mentioned ice forces are termed *global* ice forces, since they pertain to the overall forces acting on the structure. For the design of individual members, such as ice walls and bracing walls, the design pressure has to be computed independently. This ice pressure is termed *local ice pressure*. A method for evaluation of ice pressures has been developed, and the results have been presented in the form of a generalized local-ice-pressure curve. A detailed description has also been developed to guide the designer in selecting the appropriate design pressure for various members of the platform.

Production Structure Concepts

This section of the study involves the description of various production structure concepts that have been proposed to date. A detailed description of the requirements of a production structure have also been listed in this section. The basic requirements for a production structure in the arctic region includes the ability to withstand the extreme ice and wave environment and the capability for deployment and installation. Further, since the open water construction season in the arctic is only 10 to 60 days per year, and the cost of operations in the arctic is excessive, special measures need to be taken to ensure the practicality and economy of the design.

Another major requirement for a production structure is compatability with top-side facilities. A major portion of the cost of a platform is invested in these facilities. Hence, it is important to design the platform such that the costs of top-side installation, hook-up, and testing are minimized.

Detailed cost considerations in production structure design have been explained in detail in a separate section of the report.

Reliability Assessment of Design

The inherent randomness and the lack of a precise data base for ice properties brings up a very critical question in the design of production structures for Alaskan waters: What is the reliability of the design? This

question is best answered in terms of the statistical probability of failure. The assessment of the reliability is not a trivial matter. Usually the design codes incorporate the uncertainty by providing load and material factors. For arctic systems, there is as yet no special code, although a number of guidelines have been developed and standards are under preparation in Canada. Thus, use of factors from existing codes becomes of questionable validity. Therefore, this study has made an attempt to present a method of assessing reliability of designs and preparing appropriate load and material factors for use in the design of arctic systems.

Marine Operations

Since the production structures for Alaska will typically be constructed in warm water ports and then towed across the Pacific Ocean to the site, they will act as floating vessels during this tow period. The structure will have to be designed as a ship in addition to being designed for its on-site performance. Hydrodynamic behavior evaluation involves calculation of motion characteristics in terms of response spectrum for all six degrees of freedom. For irregularly shaped structures such as cones, stability during installation may be especially critical. Complete description of the techniques to evaluate the hydrostatic and hydrodynamic behavior have been presented along with examples. Damaged stability, with a flooded compartment, must also be evaluated. The towing forces required and behavior under tow must be investigated for both deep sea and shallow water conditions, the latter with the added problems of floating ice fragments and sheet ice.

Foundation Design

The design of foundations for the Alaskan Arctic Ocean poses a number of unique problems. Subsea permafrost, overconsolidated silts, methane-charged sediments, and extremely weak silty clay soils are all present in the arctic and must be adequately considered from the standpoint of bearing and sliding stability.

As a result of the frequent gouging of the seafloor by deep-keeled ridges, seafloor has typically been deeply scoured. Founding structures on such soils requires special care in design and construction. In some instances, the foundation soil may be required to be artificially strengthened. Special problems associated with foundation design have been discussed and various methods of designs outlined. Also included are state-of-the-art methods for improving foundation soils.

Design of Peripheral Ice-Resisting Wall

Since the outer walls of the structure will be subjected to high intensity loads due to impacting ice features, they require special designs. The wall has to be designed to resist the design load and also to possess high ductility under overloads, in order to inhibit progressive collapse.

The internal framing system that supports the exterior wall has to be designed so that it transfers the load efficiently to the foundation and, in the event of overload, distributes the load to neighboring structural elements without causing progressive collapse.

A detailed discussion of the design requirements and procedures has been presented in the final report.

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Ocean Wave-Induced Effective Stresses in an Elasto-Plastic Seafloor

University of California, Davis
R/OT-13
Project Initiated: October 1, 1985
Project Completed: September 30, 1986

C. K. Shen

The stability of a seabed under wave action has been the subject of a number of recent studies. Among them are theoretical analyses using poro-elastic (e.g., Yamamoto, 1983) or visco-elastic (e.g., Dalrymple and Liu, 1978) soil models, laboratory investigations of soil under rotational shear loading (e.g., Yamada and Ishihara, 1983), and wave tank experiments or *in situ* measurements of pore water pressure under wave loading (e.g., Bea et al., 1983; Clukey et al., 1984). Discrepancies seem to exist between available predictions and experimental results.

It is generally agreed that the inclusion of rotational shear loading (e.g., Ishihara and Towhara, 1984) induced by travelling waves is essential in studying the seabed response of wave loading. The present study offers a theoretical approach to analyze the wave-induced seabed stability by incorporating the recently developed bounding surface plasticity model for sandy soils into a general two dimensional finite-element program "SAC2". Since the plasticity model can adequately describe the generation of pore water pressure under different cyclic stress paths in a multi-dimensional stress space, a more realistic prediction of wave-induced seabed soil response may be achieved.

Accordingly, the study is focused on the solution of the ocean wave-induced effective stresses in seabed soils including:

(1) Adopting a plane strain elasto-plastic constitutive model to describe the seafloor soil response to wave loading,

(2) Modifying a computer program suitable for travelling wave loading and the calculation of induced pore water pressure in the seafloor soil,

(3) Performing a preliminary study to examine the importance of pertinent variables affecting the change in effective stresses in seafloor soils,

(4) Proposing possible future research toward a comprehensive solution for the design and analysis of seafloor stability under wave loading.

The study covered a period of 12 months, October 1, 1985 to September 30, 1986.

Hypoplasticity Soil Model

Discrepancies between experimental results and theoretical solutions of seafloor soil response to wave loading in part stem from the lack of understanding of the interactive mechanism of soil and fluid, and the inadequacy of mathematical modeling of soil behavior under wave loading. For instance, the poro-elastic model proposed by Yamamoto (1983) does not take into consideration energy loss, deviatoric stress-related volume change and the degradation of soil strength with pore water pressure buildup. Therefore, it seems clear that any attempt to analyze wave-induced effective stresses in seafloor soil should adopt an elasto-plastic soil model capable of incorporating rotational shear loading and deviatoric stress-induced volume changes in the analysis.

A constitutive model for granular soil based on the concept of bounding surface plasticity has recently been developed (Wang, Dafalias, and Shen, in prep.). In its present form for isotropic material, the model is capable of predicting drained and undrained behavior of sand in multi-axial stress space under both monotonic and cyclic loadings including rotational shear loading and principal stress rotation. While a well developed theoretical model will enable us to study the behavior of sand under complex loading in a multi-dimensional stress pace, it could also significantly improve our analytical capability to assess realistically seafloor stability under wave-induced loading.

The two major novel features of the model are the flow rule for deviatoric plastic strain increment and the incremental equation for the rate of pore water pressure generation (or plastic volumetric strain). In each instance, the determination of the plastic strain increment depends not only on the stress state and internal variables, as is done in modern plasticity, but also on the direction of the stress increment (not its magnitude). Such an incremental non-linear plasticity formulation is called hypoplasticity by Dafalias (1986). Good agreement was obtained when theoretical predictions were made with the model and compared with Yamada and Ishihara's (1983) true triaxial laboratory results.

2-D Finite Element Analysis ("SAC2" Program) and Model Code ("Sand" Subroutine)

A set of computer programs "EVAL" (one-element-calibration code), "SAC2" (plane strain analysis), and "SAC3" (three-dimensional analysis) have been developed by Herrmann and Mish at University of California, Davis (Herrmann, and Mish, 1983; Mish and Herrmann, 1983), which are aimed at the finite element analysis of soil structures. They are limited to small deformations and displacements and classical consolidation theory. The codes made use of the comprehensive bounding surface plasticity constitutive model for cohesive soils

originated by Dafalias (Dafalias and Herrmann, 1982). This tool has already been used to solve boundary value problems for cohesive soils. More recently, an improved numerical algorithm for the evaluation of the bounding surface for cohesive soil was developed (Herrmann, et al, 1986). In their numerical implementation of the constitutive model, the calculation of stress and strain increments is accomplished by global and local interations and includes a subincrementing process. It is valid for input data of any combination of stress and strain increments.

The newly developed bounding surface hypoplasticity model for sandy soils (cohesionless soils) has been implemented into a numerical code, i.e., a one-element analysis program "EVALS," which follows the logic and iteration procedure developed in "EVAL" for cohesive soil (Herrmann, Kaliakin, and Dafalias, 1983). Because of the incremental nonlinearity between the strain increment and the stress-increment direction in hypoplasticity formulation, the convergence of the iteration process sometimes did not occur rapidly. In addition to the above mentioned iteration and subincrementing developed in "EVAL," a filter technique has been adopted to the numerical code for sand to ensure convergence. The filter technique appears to be an efficient tool for the numerical implementation of the hypoplasticity type of constitutive models when convergence is of concern.

The subroutine "SAND," used in "EVALS" for the evaluation of the new soil model has also been incorporated into "SAC2," so that now "SAC2" is capable of modeling elastic, cohesive, as well as cohesionless soils. The matrix for the increment stress-strain equations for sand is not symmetric due to the non-associated flow rule; it is separated into symmetric and antisymmetric parts before return to the parent program "SAC2," and the antisymmetric part is included in the load matrix through the iteration process.

The governing equations in "SAC2" consist of Mass Conservation, Water Flow, Force Equilibrium, and Strain-Effective Stress Relations. For details concerning "SAC2," the readers are referred to references cited previously.

Solutions of Effective Stresses in Seafloor Soils and Comparisons

2-D finite element analyses to determine the seafloor soil response to wave loading were carried out using the "SAC2" program. Three different soil models representing the seafloor deposits were assumed, namely, the linear elastic model, the "overburden" dependent elastic model, and the hypoplasticity model. In the analysis, only harmonic travelling waves are considered, the seafloor is assumed to be horizontal, and a portion of length equal to the wave length is considered. Appropriate boundary conditions are specified for the studies. The various input parameters for each soil model are given in Table 1. The 2-D finite element mesh is discretized by 144 (12 x 12) rectangular elements to represent the sand deposit. In each of the 12 sand columns, the spacing ratio between nodes is a constant of 0.8 from bottom to top. Furthermore, the water tank experiment results from Cornell University were compared with the finite element predictions using both the linear

Table 1. Input Parameters for Finite Element Analyses

Geometry and Wave Form		Water Depth: 20^m, Wave Height: 2.5^m; Wave Period: 15^s, Wave Length: 197.4^m
Soil Model	Linear Elastic	Bulk Density: 1.5 t/m^3 , Poisson's Ratio: 0.3 Permeability: 1×10^{-4} m/s Young's Modulus: 1.31×10^4 kN/m^2
	Elastic (Overburden Dependent Modulus)	Same as in the linear elastic case except that moduli are not constant. $G = G_e P_{atm} \frac{(2.975-e)^2}{(1+e)} \left(\frac{\sigma_v'}{P_{atm}}\right)^{1/2}$ kN/m^2, $G_e = 100$
	Hypo-plasticity	Conventional sand property: $\lambda = 0.14$, $\kappa = 0.01$, Critical state line: $M_c = 1.47$, $M_e = M_c$ Phase transformation line: $M_{cp} = 1.24$ Elastic shear modulus index: $G_e = 100$ Model Parameters: Ref (e.g. Wang, et al., 1985) Plastic shear modulus index: $H_{rp} = 0.1$ Plastic index on flat cap: $Z_{et} = 1.0$ Shear compaction parameters: $A_c = 2.5$, $A_e = 2.5$, $B_c = 4.5$, $B_e = 4.5$

elastic and the hypoplasticity soil models. Based on the above mentioned investigation, tentative conclusions may be stated with respect to the adoption of the hypoplasticity soil model in seafloor effective stress calculation under wave loading.

(1) The amplitudes of the wave-induced deviatoric stress are slightly smaller than the corresponding multi-layered elastic solution values for layers near the seabed surface due to soil plasticity.

(2) Excess pore water pressure can be generated by the rotational shear stress path. If it is not dissipated, it will be accumulated and will build up a residual pore water pressure in the seabed soil.

(3) It has been demonstrated that the stress paths in the deviatoric stress subspace are elliptical under harmonic wave loading for both "overburden dependent" elastic and hypoplasticity soil materials.

(4) The failure criterion based on "stress angle" (Yamamoto, 1977) is suitable for seabed instability analysis, because, in rotational shear, the deviatoric stress amplitude does not decrease to zero before it reaches failure. The "stress angle" increase due to the increase in residual pore water pressure thus may lead to failure. This "time dependent" ultimate state and failure cannot be predicted by elastic or viscoelastic soil models.

(5) In comparison with the water tank experiment, the plasticity analysis can better predict the test results than can elasticity analysis.

Conclusions

The current study has demonstrated for the first time that a hypoplasticity constitutive soil model can be successfully applied to describe the soil response under travelling wave loading. Preliminary results have shown that the inclusion of rotational shear effect in the constitutive model can indeed improve the prediction of induced effective stresses in seafloor soils. This has been substantiated by re-analyzing the water tank experiment conducted a Cornell. We therefore believe that additional research along this line should be continued to develop its full potential with the objective of establishing basic guidelines for the evaluation of ocean floor stability and the design of ocean front and offshore structures.

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Structure of the Hosgri Fault Zone at Depth: A High Resolution View and Computer Graphics Models

University of California, Santa Barbara
R/OT-15
Project Initiated: October 1, 1985
Project Completed: December 31, 1986

Bruce Luyendyk

The Hosgri Fault Zone is a major seismogenic zone found offshore along the coast of central California (Figure 1). Near Santa Maria the zone crosses through the offshore Santa Maria Basin where extensive oil production is taking place. Petroleum geologists have suggested that the Hosgri is a thrust fault and that the fault zone dips northeast and flattens at depth (Crouch, Bachman, and Shay, 1984). This would be a considerably more dangerous geometry for seismic hazard than if the faults were vertical. This fault zone and related ones may be hypothesized to underlie such critical sites as the Diablo Canyon Nuclear Power Plant, the Space Shuttle installation at Vandenberg Air Force Base, and the Liquified Natural Gas Facility near Point Conception. Our goal has been to map and understand the Hosgri Fault Zone and associated faults at depth in the region offshore Santa Maria. We hope to understand the structural and tectonic history of the area and its relation to the history of the Transverse Ranges to the south.

The direct benefits of this project to government, industry, and university scientists are an improved understanding of the deep structure of the fault zone with its impact on hazards and resources offshore, a tectonic history of the region relating the structures of the Coast Ranges and Transverse Ranges, computer graphics methodology to view complex geologic structure, and more informed planning and decision making by government and industry for resource development.

The overall project objectives were to (1) map the structure of the Hosgri Fault Zone at depth; (2) interpret this structure in terms of geologic hazards and tectonic history; (3) develop and implement new technology for viewing geologic structure in three dimensions using computer graphics that may be readily implemented by other academic researchers; and (4) to inform researchers in universities, the oil industry and government agencies of the results of our work. This grant was for a one-year feasibility study.

The Hosgri Fault Zone

The San Gregorio-Hosgri (or simply Hosgri) Fault Zone extends over 120 km along the coast of central California from near San Francisco on the north to the Santa Maria basin on the south (Figure 1). It is an active fault (Leslie, 1981) similar to the San Andreas system. The fault is believed to have accommodated significant right slip during the Tertiary Period (Silver and Normark, 1978). Displacement estimates are near 115 km (Graham and Dickinson, 1978), 97 km (Hall, 1981), and 110 km (Dickinson, 1983), although Hamilton and Willingham (1977) estimated less than 20 km. Movement on the fault is believed to have begun in middle Miocene time (Graham and Dickinson, 1978), and has been related to the clockwise rotation of the Transverse Ranges (Luyendyk, Kamerling, and Terres, 1980; Luyendyk, et al., 1985; Hornafius, 1985).

Interest in this fault has increased since the 1970s because of several proposed coastal and OCS (Outer Continental Shelf) developments, including the Diablo Nuclear Power Plant near San Luis Obispo and oil development in two offshore basins that are astride the fault near its north and south ends. Near the southern Hosgri fault, oil exploration has focused on the offshore Santa Maria Basin which comprises a NW-SE elongate thick accumulation of Neogene sediments (mostly Monterey Formation) bounded by the Hosgri Zone on the east and the Santa Lucia high on the west (Howell et al., 1978).

There is no doubt that the Hosgri fault poses a serious seismic risk. Smith (1974) estimated the fault to be capable of M 6.5, from its mapped length. In 1927, the third largest earthquake in California during this century occurred offshore from the Santa Maria Basin. With an estimated magnitude of 7+, it has been located on the Hosgri fault (Gawthrop, 1978) or close by (Yerkes et al., 1981). Focal plane solutions suggest present motion on the Hosgri is right-oblique slip on a NE to E dipping fault plane (Gawthrop, 1978).

Work on Project R/OT-10. The goal of this Sea Grant project (which preceded R/OT-15) was to describe the shallow structure of the southern terminus of the Hosgri Fault Zone offshore south central California, and to interpret this structure in terms of geologic history and geologic hazards. The study area ranged from a few kilometers south of Point Sal to south of Point Conception, and from the coastline to approximately 10 km offshore (Figure 1).

We used new data sets to map the fault zone in this study area. These data are high resolution 1.0 second seismic reflection records obtained for geohazards evaluation. Besides the use of traditional interpretation and mapping techniques with these data, we developed a software system to construct computer graphics 3-D geologic models of the structure beneath the seafloor.

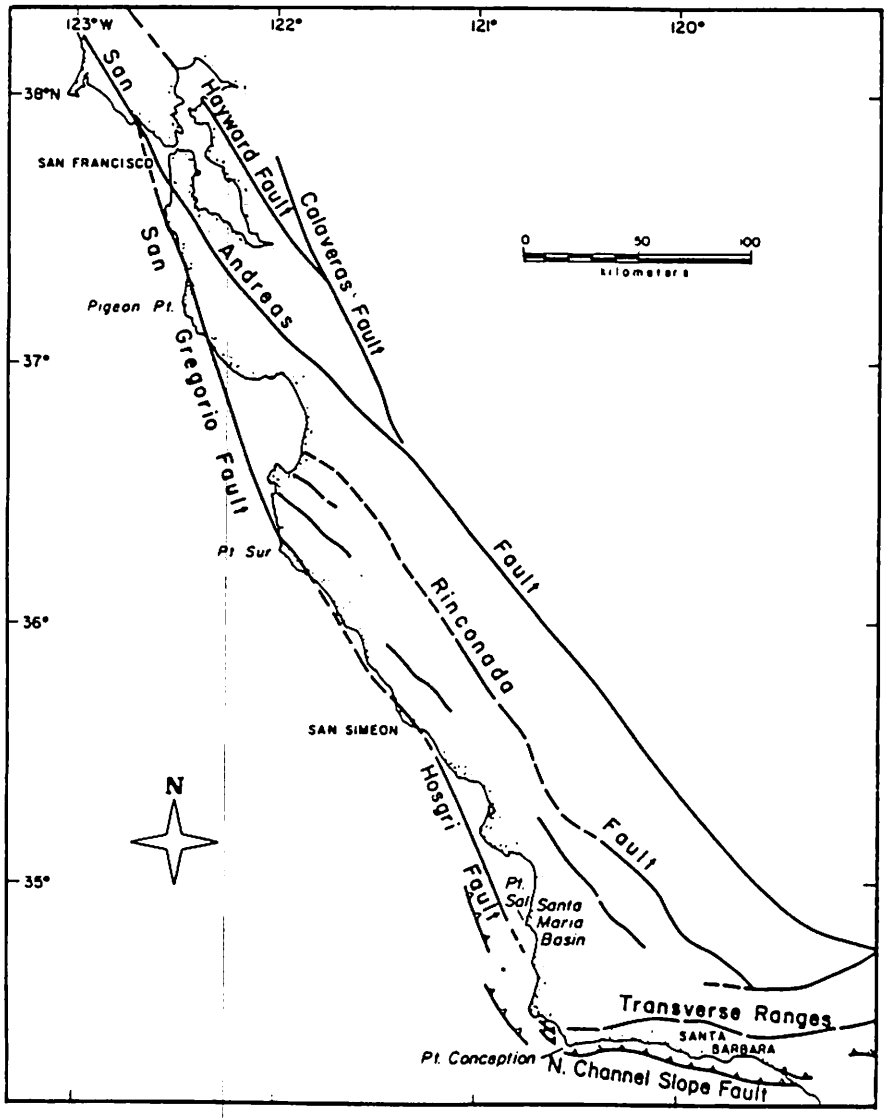

Figure 1. Fault map of the California Coast Ranges showing the trend of the Hosgri Fault Zone. (From Crouch, Bachman, and Shay, 1984).

Our interpretation and mapping effort has shown that the east-west trending Transverse Ranges faults such as the Lompoc-Solvang or Santa Ynez River Fault (Sylvester and Darrow, 1979; Hall, 1981) do not extend a significant distance offshore; at least in the section above 1.0 seconds (RT) subbottom. The Hosgri Fault Zone is expressed as a single fault trending nearly north–south about 5 km offshore. We have traced it south of Point Arguello in general agreement with the extrapolations of McCulloch et al. (1977).

Is the Hosgri fault zone primarily a thrust rather than a strike-slip zone? A paper by Crouch, Bachman, and Shay (1984) presented interpretations which indicate that the Hosgri and associated faults offshore from the Transverse Ranges appear to have the form of thrust faults below 1.0 seconds reflection time. They used high resolution seismic reflection data from Nekton, Inc. to produce these interpretations. Their data coverage includes the northern Santa Barbara Channel shelf and the region encompassing the Hosgri Fault Zone north of Purisima Point, the offshore Santa Maria Basin.

In both the offshore Santa Maria Basin and the northern Santa Barbara Channel the interpretations of Crouch, Bachman, and Shay (1984) show thrust faults which are "commonly imbricate and curve asymptotically downward to a basal sole thrust." In addition, they map fold axes as parallel to fault traces rather than en-echelon and oblique to the fault trends as might be expected in wrench tectonics. This last observation implies that the present predominant motion on the Hosgri zone is thrusting rather than dextral slip.

Summary of Results

Our approach to the problem was threefold: (1) to reprocess existing seismic reflection data to reveal deep structure, (2) to obtain industry seismic data in the vicinity of the fault zone, and (3) to construct 3-D color computer graphics models of the subbottom structure to reveal interrelationships of complex structural parameters.

We performed reprocessing of USGS Data Set PA 18254. These data include 4,444 line kilometers of 12-channel minisleeve seismic exploder data. These data were originally recorded to 2.0 seconds but only processed to 1.0 seconds. We obtained field tapes of the data from the Minerals Management Service in Los Angeles, and 4 lines were selected for reprocessing.

The first step was to demultiplex the field tapes which were recorded in an unknown version of SEGB format. After some futile experimentation at Lawrence Berkeley Labs, it was decided to have the tapes reformatted to SEGY by Oil Data Inc. in Houston, which was one of the original processing contractors.

This step was successful and allowed us to experiment with reprocessing the SEGY tapes at University of California, San Diego, Scripps Institution of Oceanography, using the processing system SIOSEIS. We experimented with various stacking velocities, filters, time-varied gains, etc. We found that reliable reflections were visible only rarely to 1.5 seconds reflection time. We concluded that this approach was unfruitful for studying

the deep structure of the Hosgri Fault Zone.

As a result of the downturn in the oil industry, Nekton Inc. was able to release to us some very high quality industry multichannel reflection data for the cost of reproduction. These data comprise 45 seismic lines of which 21 are 2.5 second, 24 fold, 80 KJ sparker; 19 are 3 second, 36 fold, 800 cubic inch watergun; and 5 are 4 second, 96 fold, 1600 cubic inch watergun. They are located over the Hosgri Fault Zone in the vicinity of Point Sal (Figure 1).

Using eight sparker lines and four 4.0 second watergun lines, we mapped the structure of the Pliocene-Miocene unconformity near the intersection of the Hosgri and the Lion's Head fault west of Point Sal (Figure 2). Deep reflection data effectively mapped the association of folding and faulting west of the Hosgri. Although folding extends through the entire section, faulting just west of the Hosgri is often restricted to below early Pliocene age rocks. The area around the Point Sal–Lion's Head fault is structurally uplifted along large west to northwesterly trending faults and folds, resulting in increased east-side-up vertical offset on the Hosgri Fault Zone. Much of the mid-Pliocene to Miocene section mapped west of the Hosgri is apparently missing to the east of the Hosgri (Figure 2) indicating local uplift and wave-cut erosion east of the Hosgri near the Lion's Head fault. Right slip on the Hosgri perhaps on the order of a few km could be effectively dissipated by reverse faulting on the Lion's Head fault. Focal mechanism solutions by Eaton (1984) show a N39E dipping reverse fault solution on strike with the Lion's Head fault near its intersection with the Hosgri. In this study area the Hosgri was interpreted as a vertical fault to the depths mapped (10,000 feet at a velocity of 5000 ft/sec). Lesser faults immediately to the west of the Hosgri do have a component of dip to the east and are interpreted as thrust faults.

We also constructed a 3-D color graphics model of the area mapped

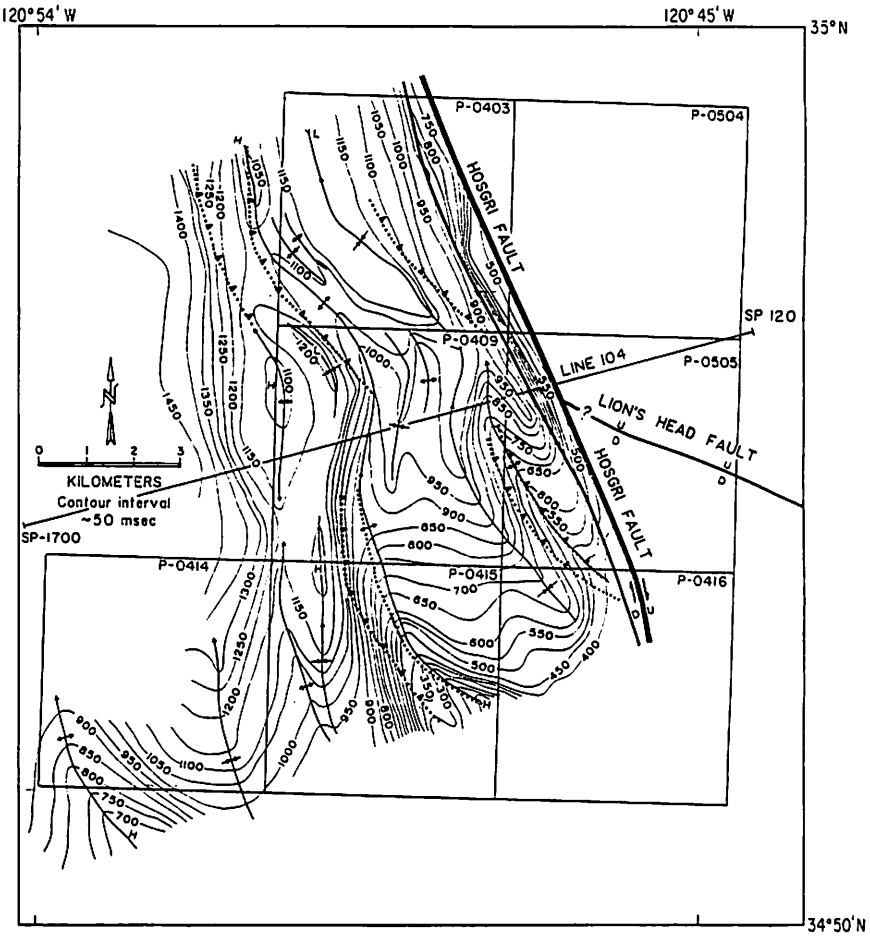

Figure 2. Structure map of Pliocene-Miocene unconformity, lease blocks 403, 504, 409, 505, 414, 415, and 416 west of Point Sal (Figure 1). Contour interval is 50 Msec of two-way travel time. Dotted faults are mapped beneath the Pliocene-Miocene unconformity. Reverse faults are shown with teeth on the upthrown side.

in Figure 2 (Figures 3, 4). We started by digitizing the faults and unconformities and merging these data with navigation. These interpreted surfaces were then gridded. The software we used to construct the 3-D models is built around DI 3000 from Precision Visuals Inc. However, we had to make modifications to allow the display of discontinuous surfaces, an attribute DI 3000 cannot handle. The models are displayed with an interactive software package we designed, which allows selection of colors, viewing elevation, and viewing direction. A Tektronix 4113 intelligent color terminal is used to view the models. This terminal permits the local storage of the models on 8-inch floppy disks. Additionally, the user can zoom or pan on the model image.

In the models shown in Figures 3 and 4 the viewer is looking from the northwest to the southeast parallel to the strike of the Hosgri Fault Zone. Minor faults parallel the Hosgri and the more westerly ones dip toward it. The Pliocene-Miocene unconformity is strongly folded with axes also paralleling the Hosgri trend. The associated minor faults do not cut the unconformity whereas it is terminated against the Hosgri Fault Zone.

Conclusion

We have learned that there are little or no data in the public domain that can be studied to determine the deep structure of the Hosgri Fault Zone. Even after obtaining high quality deep-penetration industry data, we still see the problem as complex. In the area we mapped,

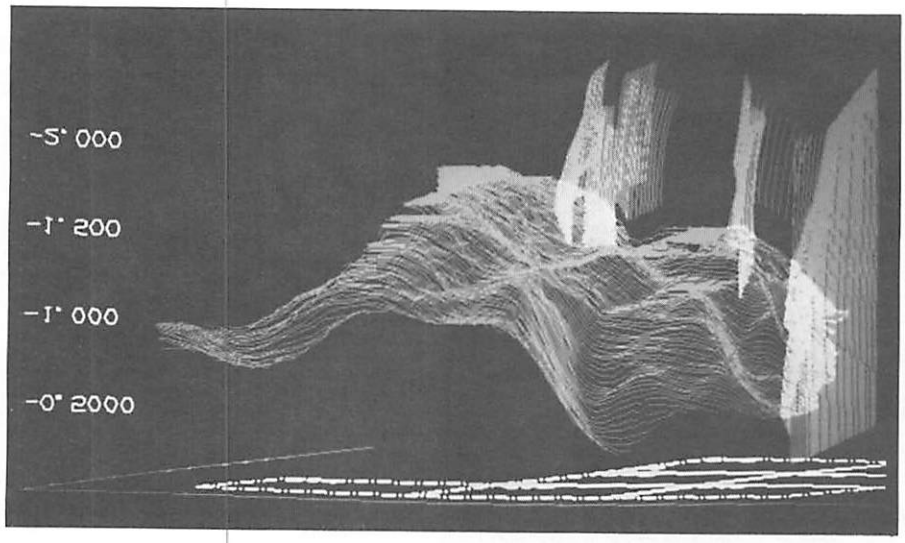

Figure 3. A graphics model of the area mapped in Figure 2. The Pliocene-Miocene unconformity is shown as horizontal lines. The boxes are tract boundaries (Figure 2). The vertical scale is in seconds (two-way). Vertical exaggeration is about 4, assuming a sound speed of 5000 ft/sec. Ticks on the horizontal axis are at one kilometer intervals. The view is from the northwest toward the southeast at an elevation of 25 degrees. The Hosgri Zone is the vertical curtain-like pattern on the left.

our interpretation favors the conclusion that the Hosgri Fault Zone is a mostly vertical strike slip fault. This view strongly contrasts with that of Crouch, Bachman, and Shay (1984). During this project we met with Crouch, Bachman and Associates Inc. to review their data, some of which included our Nekton data set, and to study their interpretation. One point they argue is that a reflection from a flat dipping Hosgri fault can be seen in strike parallel lines to the east of the fault, thereby constraining the fault zone to flatten eastward from its mapped outcrop. Also, they believe the main break of the fault to be the parallel fault we mapped less than one kilometer west of our Hosgri trend (Figure 2). We take issue with both of these interpretations. We believe that the structural style mapped in the south during project R/OT-10 and in this study area, along with earthquake focal plan solutions, favor an interpretation of right oblique slip motion for the Hosgri Fault Zone.

Cooperating Organizations

U.S. Minerals Management Service, Los Angeles
University of California, Berkeley, Lawrence Berkeley Laboratory
University of California, San Diego, Scripps Institution of Oceanography

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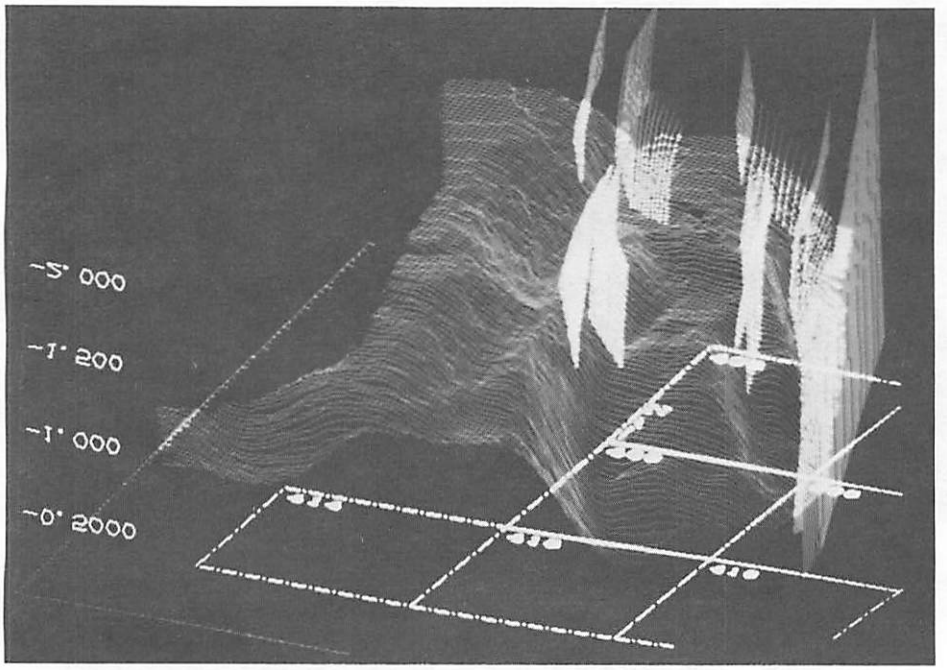

Figure 4. Same as Figure 3 with an elevation of 3 degrees.

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Marine Affairs

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Comparative Study of Dungeness Crab Fisheries

University of California, Davis
R/MA-20
Project Initiated: October 1, 1983
Project Completed: September 30, 1985

Louis W. Botsford

In the second year of this study we have focused on a potential environmental mechanism (wind-driven larval transport) revealed in the first year and its interaction with density-dependent mechanisms that may be involved in cycles in Dungeness crab populations along the Pacific coast. This analysis has led to development of a coastwide model of the ways in which oceanographic conditions may interact with population dynamics. Fieldwork to evaluate portions of the model has involved preliminary sampling of both larval and post-settlement phases. In collaboration with others, we plan to extend this work to a coastwide collaborative investigation of Dungeness crab population dynamics.

Analysis

Part of the effort this year was spent on reevaluation of the correlation revealed last year between spring wind stress and crab catch. Rather than focusing on only one wind direction, we examined correlations in all possible wind directions. The results showed generally high correlations between catch and southerly wind directions at the appropriate lags (Table 1). There are several ways in which this correlation could reflect wind-driven larval transport southward and onshore.

Since the essential purpose of this project was to clarify the ways in which coastwide environmental conditions and density-dependent recruitment could lead to cyclic behavior, we needed to understand the interaction between environmental forcing and density-dependent recruitment in populations in general. Environmental influences on recruitment have been identified as potential causes of fluctuations in abundance of several fish and crustacean populations. Since the importance of environmental forcing is usually demonstrated by statistically comparing a time series of environmental data with a time series of catch or abundance data, an essential question in evaluating environmental mechanisms is, What is the expected relationship between the environmental series and the catch or abundance series? We analyzed the relationship between a time series of an environmental forcing variable and a time series of catch or abundance for a fishery in which a potentially nonlinear environmental forcing mechanism affects density-dependent recruitment and the population is fished at a constant rate. A density-dependent recruitment mechanism will be most sensitive to frequencies in the environmental time series near the frequency of unstable cycles [i.e., approximately 1/(2 x mean population age)] even for stable systems. The effect of harvest is to reduce higher frequencies. Both of these depend on harvest rate and the width of cohort size distributions (for a size-selective fishery). Application of the results to the northern California Dungeness crab show that fluctuations in spring winds alone cannot cause the observed catch, and that a nonlinear effect of wind and/or density-dependent recruitment are possible causes of the observed cyclic fluctuations. These results were also related to other invertebrate populations and were received with great interest by other crustacean researchers from the United Kingdom, Australia, and Canada at the Lobster Recruitment Workshop in New Brunswick in June 1985.

This single population model has been expanded to include variation in numbers along a spatial dimension (i.e., north–south along the coast). This model is currently being used to determine the coastwide catch records that would result from scenarios such as random diffusion of larvae between benthically isolated populations or north–south and onshore transport driven by the winds. For the former we can determine the levels of density-dependence and dispersion necessary for synchronous cycles. The latter is an extremely complex issue, and no simple, general results are yet available.

Further work on the egg predator worm this year resulted in clarification of the potential role of the worm in conjunction with other factors. The worm could be involved in the cycles only in conjunction with other mechanisms (i.e., another density-dependent recruitment mechanism or environmental forcing). The existence of multiple equilibria (i.e., low worm/high crab states and high worm/low crab states such as in central California) also require the presence of a second density-dependent mechanism.

Field and Laboratory

Because ongoing work indicated more attention should be paid to the larval stages, we expanded the focus of our fieldwork to include the late larval stages and did our sampling off Bodega Bay (to increase the number of possible trips). We were able to make five trips to sample vertical and onshore–offshore plankton distributions and eight trips for benthic samples. We were fortunate in having an unusually large settlement near Bodega Bay this year.

The vertical distribution of Dungeness crab larvae is poorly understood yet of critical importance in establishing the mechanisms by which oceanographic conditions

influence successful settlement. Our preliminary results showed megalopae distributed throughout the water column during the day and only in the neuston at night. This is not consistent with the conclusion of the California Department of Fish and Game that megalopae do not migrate vertically (Reilly, 1983), but it is consistent with work done off British Columbia (Booth et al. 1985).

We have continued to obtain plankton samples from the joint NMFS/USSR cruises. This year's samples are currently being analyzed.

Benthic samples were taken for information on cannibalism, predation, and crab distribution and abundance. We found evidence of juveniles in the guts of crabs of all sizes sampled (i.e., up to 140 mm carapace width). Cannibalized crabs appeared to be young-of-the-year. There was substantial predation on early benthic stages (and a few megalopae) by fish species such as tom cod (*Microgadus pacificus*), staghorn sculpin (*Leptocottus armatus*), smelts (family Osmeridae), and cabezon (*Scorpaenichthys marmoratus*). Newly settled crabs were initially found out to the 30-fathom isobath, but the distribution contracted to the area inshore of 10 fathoms as abundance declined from extremely high levels.

Because the shape of the cohort size distribution is critical in determining growth rates from multiple cohort size distributions (Botsford, 1984 and Botsford, in press), we are conducting a small growth experiment (40 postlarval crabs) to determine the variability in growth of crabs of the same age. This study has indicated the possible presence of a waterborne growth inhibitor similar to that found in lobsters (Nelson et al., 1983). If this effect is present in the natural environment, it could have significant implications for population dynamics as well as the practice of determining growth rates from large year classes.

Collaboration

Because of the coastwide extent of cycles in catch and the potential for coastwide dispersal of the larval phases, we have increased our contact with other researchers in Oregon, Washington, and British Columbia. In March 1985 we held a meeting of oceanographers and biologists with expertise in Dungeness crab biology and mechanisms by which physical processes in the California Current can influence planktonic larvae. Attendees included D. Armstrong (University of Washington), B. Hickey (University of Washington), G. Jamieson (Pacific Biological Station, Nanaimo, B.C.), E. Pikitch (Oregon State University), T. Powell (UC Davis), J. Shenker (UC Davis/Bodega Bay), and P. T. Strub (Oregon State University). The consensus of this group was that after preliminary data on such basic issues as vertical distribution of larva was in hand, proposal of a joint, collaborative effort to study the interaction between physical and biological processes would be in order. Accordingly, we are currently pursuing the required baseline data and plan such a proposal effort within the next year.

We have also maintained close contact with the Pacific Fisheries Environmental Group of NMFS in Monterey, and Botsford presented a seminar there in September 1985.

Cooperating Organizations

Oregon State University, Newport, Oregon
Pacific Biological Station, Nanaimo, B.C.
Pacific Fisheries Environmental Group, National Marine Fisheries Service, Monterey, California
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Table 1. Cross Correlation Between Wind Stress in Each of Eight Directions and Crab Catch in Washington (NOR), Oregon (CEN), Northern California (ECC) and Fort Bragg (FB).

		Direction (degrees)							
	Lag	90.0	112.5	135.0	157.5	180.5	202.5	225.0	247.5
NOR	3	-0.168	0.038	0.161	0.220	0.254	0.277	0.291	0.278
x	4	-0.072	0.315	0.479*	0.532*	0.547*	0.540*	0.503*	0.389*
wind	5	0.071	0.425*	0.538*	0.555*	0.540*	0.506*	0.437*	0.281
45°N	6	0.125	0.267	0.286	0.270	0.244	0.211	0.158	0.060
CEN	3	-0.326	-0.105	0.121	0.219	0.270	0.304	0.331	0.351
x	4	-0.443	-0.061	0.270	0.399*	0.460*	0.497*	0.522*	0.527*
wind	5	-0.159	0.293	0.507*	0.539*	0.531*	0.508*	0.468*	0.384*
42°N	6	0.156	-0.624*	0.690*	0.616*	0.537*	0.455*	0.353	0.186
ECC	3	-0.073	0.297	0.444*	0.451*	0.431*	0.402*	0.359	0.276
x	4	-0.253	0.212	0.475*	0.537*	0.549*	0.542*	0.517*	0.453*
wind	5	-0.027	0.303	0.414*	0.407*	0.382*	0.349	0.303	0.220
42°N	6	0.350	0.456*	0.318	0.196	0.190	0.034	-0.049	-0.165
FB	3	-0.208	0.101	0.239	0.304	0.344	0.376*	0.402*	0.399*
x	4	0.005	0.288	0.371*	0.396*	0.402*	0.398*	0.377*	0.291
wind	5	0.397	0.343	0.239	0.165	0.102	0.036	-0.053	-0.206
39°N	6	0.358	0.304	0.208	0.141	0.085	0.025	-0.055	0.191

*Indicates significance at the 0.05 level (uncorrelated series test).

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A History of Maritime Cargo Containerization in the California Maritime Industry: The Case of San Francisco, 1945–1970

University of California, Santa Barbara
R/MA-23
Project Initiated: October 1, 1984
Project Completed: September 30, 1986

Donald T. Fitzgerald and Carroll W. Pursell

When humans first began transporting cargo in boats, they loaded it into and out of cargo spaces by hand. While boat and ship construction changed from wood to steel, and methods of propulsion from wind to steam, stowing cargo in ships' holds remained heavily dependent on manual labor.

In the late 1950s, however, a new mechanized method of cargo handling was introduced on the waterfront. Large steel containers arrived at the docks already packed with up to twenty tons of cargo. These containers were lifted by giant cranes into the holds of specially designed containerships and unloaded at their destinations without the cargo having been touched by human hands. The once familiar waterfront scene of longshoremen manually loading cargo into and out of ships has all but disappeared, replaced by a highly mechanized system. The new technology of containerization completely reordered the maritime cargo transportation industry.

While California ports at Oakland and Los Angeles have become major container terminals, the Port of San Francisco has not made the full transition to this specialized technology. This study examines the manner in which the maritime community of the Port of San Francisco dealt with the challenges and problems created by containerization. In so doing it examines the relationship of this technology to the community of which it became a part, describes how three segments of this port's community dealt with the new system, and examines what affected the decisions the port made in dealing with the problems created by this technological change.

The Port of San Francisco's post-World War II attempts to modernize its facilities had been hampered by the philosophy of the state legislature, which had operated the port for the past 100 years. Having seized the port in 1863 from the chaos existing under ownership by the city of San Francisco, the state operated it with little emphasis on updating its facilities as long as there was a sufficient number of other ports in the Bay Area to handle the waterborne trade.

The introduction of containerization to the San Francisco Bay maritime community by the Matson Navigation Company in 1958 reemphasized the need to construct modern port facilities. City officials, wishing to control the destiny of the port that graced their city, sought a change of ownership from state to city.

In 1958 Governor Ronald Reagan signed legislation that allowed San Francisco to regain ownership of its port. Port officials no longer had to balance decisions for the port with statewide considerations, but could develop San Francisco's port in its own right. In order to raise revenue to construct new maritime facilities, the port attempted to erect nonmaritime commercial developments along the historic and scenic Embarcadero. These commercial ventures were opposed in 1960 by environmentally concerned citizen's groups, which objected to the construction of structures that would change the very nature of the waterfront as well as place a visual barrier to the view from the "City by the Bay." Port officials were required to balance concerns for the heritage of the past and environmental awareness of the present with anticipated technological requirements of the future. They chose to preserve the maritime environment of the Embarcadero, shift the maritime industry to the undeveloped southern waterfront area, and raise construction funds for new cargo terminals through public bond issues.

The port's efforts to participate in the container era were plagued with difficulties. Administrative friction caused by differences of opinion between Port Directors and members of the Port Commission led to changes of Port Directors at this crucial time. In addition, the Port Commission faced the delicate problem of having one of its tenant shipping companies purchased by the son of the mayor of the city, a situation that eventually resulted in a conflict-of-interest ruling. In the late 1960s, port authorities leap-frogged container technology and constructed a facility for a newly developed cargo delivery system using cargo lighters aboard ships, called LASH. After only a few years, however, the shipping company for which the facility was designed, Pacific Far East Lines, stopped using the LASH system and declared bankruptcy, leaving the port with a facility designed for the abandoned technology. In 1974, another effort to meet the challenges of the container era met with disastrous setbacks. The underwater foundation for a proposed container facility collapsed, either because of engineering miscalculations or an earth tremor. Whatever the cause, the facility could only be partially used for container operations.

The second element of San Francisco's maritime community examined is its home-based steamship companies. These cargo carriers were not only faced with the

developing new technology of containerization, but also with a paradoxical situation it created for them. While steamship operators had traditionally sought to reduce the cost of dockside labor, they were now presented with a cargo handling system that promised to all but eliminate the worker on the docks. The carriers, however, were faced with huge expenses necessary to convert from manual cargo handling to the new mechanized system. Steamship companies differed in their reaction to this situation. The Matson Navigation Company made a systematic study of containerization, embraced the new cargo handling system, and prospered in the new technological era. Pacific Far East Lines on the other hand, had adopted the LASH variation of containerization, a system designed for shallow-water ports. Having received financial assistance and encouragement for the LASH system from the Federal Maritime Administration, Pacific Far East convinced San Francisco to construct a facility especially for the new process. After the port constructed the LASH terminal, the shipping company abandoned LASH, changed to containerization cargo operations, and invested millions of dollars to convert its LASH fleet to containerships. Unable to survive the resulting financial drain and, according to some, the effects of poor management, Pacific Far East Lines collapsed into bankruptcy. San Francisco was left with a facility specially constructed for a now-abandoned system of cargo delivery.

The third element of San Francisco's maritime community to be examined in this study, the West Coast-based International Longshoremen's and Warehousemen's Union (ILWU), had opposed attempts to introduce mechanization on the waterfront since the 1930s. In 1960, however, union president Harry Bridges succeeded in convincing union members to accept increased use of machines on the docks. In return, workers received a share of the financial savings carriers experienced from the increased mechanization. As one union member put it, workers now received "a bite out of the machine." The agreement between longshoremen and their employers was hailed as a new era of labor-management cooperation. Ten years later, however, changing social and economic conditions led longshoremen to return to their old weapon against employers, the waterfront strike.

The history of the Port of San Francisco highlights the complexity of the relationship between a technology and the community with which it interacts. It reveals that technological advancements are not inevitable, but rather that they occur as a result of a series of choices, often nontechnological, made by that community. In addressing the challenges presented by this new technology, the Port of San Francisco included economic, social, political, and environmental considerations in deciding its choices of action.

This study of the Port of San Francisco presents only a few events in the ongoing process of addressing technological change. In the Spring of 1987, the port once again proposed to construct office buildings on the old waterfront piers. It has also embarked on plans to construct a new container freight station to meet the challenge of a container age now more than twenty-five years old. Since the port is considering some of the same decisions it considered a quarter century ago, the history of the Port of San Francisco must continue to be studied to provide a clearer understanding of the process of port development.

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Benefit/Cost Valuations in a Multi-Species Ecosystem: Implications for Management of the Southern Sea Otter

San Diego State University
R/MA-24
Project Initiated: October 1, 1984
Project Completed: September 30, 1985

Ronda K. Hageman

The U.S. Marine Mammals Protection Act [Section 2(6)] states explicitly, "marine mammals have proven themselves to be resources of great international significance, aesthetic and recreational as well as economic, and it is the sense of Congress that they should be encouraged to develop to the greatest extent *feasible commensurate with sound policies of resource management* (emphasis added)." Section 103(B) requires consideration of all socioeconomic factors, which include "utilization of fishery resources." In this regard, we have refined recreation valuation methodologies and applied them to the problem of quantifying a variety of relevant benefits and costs associated with protection of a threatened marine mammal, the southern sea otter in California.

On the cost side, post-war expansion of the otters' range has made evident the incompatibility of sea otters and commercial shellfisheries. Though sea otters do not eradicate shellfish populations, their efficient predation does virtually eliminate both recreational and commercial fisheries for abalone and sea urchins since the shellfish that remain, though numerous within otter territories, are both too small and cryptic for harvesting by human divers (Lowry and Pearse, 1974). As a result, we investigated the historical economic impact on the most controversial commercial fishery, abalone, within the context of *incremental* losses over and above those that might be attributed to human overfishing. These losses were generated using econometric techniques to project over non-otter fishing areas as compared with fishing areas within the otters' range. Results are shown in Table 1 and Figures 1 and 2. In Table 1, incremental losses are shown for the fishing industry, where the lower bound reflects price elasticity adjustments to account for actual gains to fishermen due to higher prices that would not have resulted without otter predation of abalone. However, it should be noted that this adjustment should not be made in a final loss calculation to society since the gains to fishermen are still losses to consumers in the form of higher prices.

Losses in the sea urchin industry to date could not be tabulated since commercial operations had not begun in California prior to 1971, the same time when the otter population expanded into shellfisheries south of Santa Cruz. However, because the fishery appears to be quite strong and does not seem to have begun to experience diminishing production under overfishing pressure as yet (see Table 2), it may be that any losses due to otter predation of urchins have been very small and in the form of very short-term distributive losses as fishermen found it necessary to move slightly southward out of the sea otters' range to find sea urchins in abundance.

Future losses in both abalone and sea urchin fisheries are difficult to project at this time because of a very

Table 1. Estimated Reductions in Landings and Value, Morro Bay Ports (1984 Dollars)

Year	Reduction in Landings (lbs, 000's)	Foregone Value (Historical Prices) ($ 000)	Foregone Value (Projected Prices) ($ 000)	Value of Actual Landings ($ 000)
1968	545	409	147	711
1969	731	637	263	530
1970	945	854	473	261
1971	817	765	388	291
1972	724	659	247	382
1973	789	575	397	186
1974	699	660	338	201
1975	672	717	370	143
1976	636	871	348	114
1977	594	870	317	110
1978	559	963	326	69
1979	524	901	340	26
1980	474	931	303	36
1981	397	779	271	12
1982	310	553	204	25
1983	317	566	214	20
1984	299	788	209	14
Total Present Value Foregone		$15,600	$6,542	

Reductions in landings = projected landings – actual landings. Projected landings based upon historical trends in landings for other Santa Barbara ports where sea otters were not present for the time period analyzed.

Reductions in landings x historical price in the Santa Barbara area.

Reductions in landings x price projections – actual landings x (historical price – projected price). Projected prices based upon price trends in the Santa Barbara area in post-war years up to 1968, when the sea otter take of abalone began to significantly reduce landings.

Compound rate = 2.5% per year (in real terms).

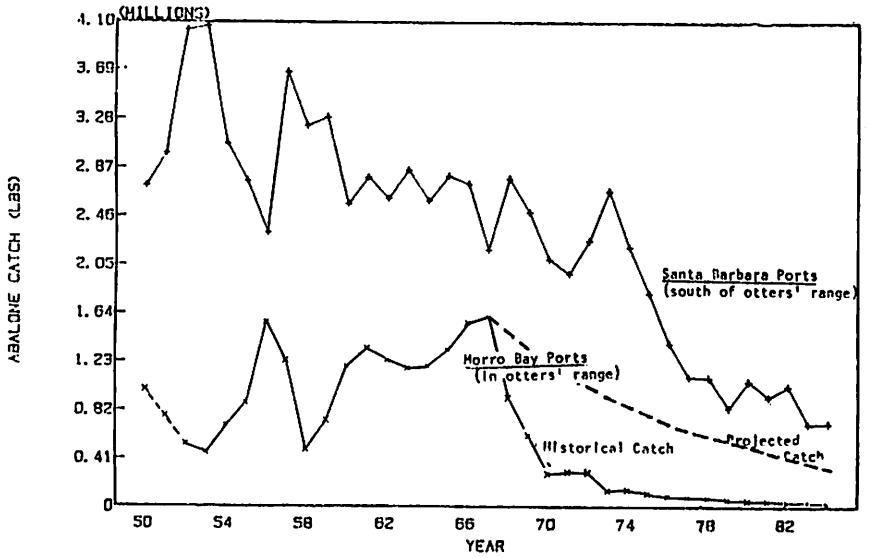

Figure 1. Abalone catch, 1950–84.

high degree of uncertainty about growth and potential range expansion of the otter population, which has been relatively stable over the last decade. As a result, no additional shellfisheries have been affected by otter predation once the controversial range expansion through the mid-1970s slowed. In recent months, a large number of incidental otter drownings in gill nets has led to legislation closing shallow-depth gillnet fishing (up to 20 fathoms) within the otters' range. As a result, a portion of this project's resources have been devoted to gathering information on the losses to the gillnet fishery, and estimates should be completed by year's end.

Another aspect of the cost calculations was suggested by researchers familiar with events during the period of rapid range expansion. Though some economic evidence exists that the incremental losses in recreational values resulting from virtual elimination of clamming at Pismo Beach were insignificant (see Holt, Estes, and Fraser, in press), it may be true that less-populated beaches suffered greater reductions in visitation. We have been gathering data from those areas previously known for abundant sport clamming for the purpose of comparing visitation over the last ten years with trends in visitation at other California state parks. The final results can be used to ascertain whether significant recreational values were lost as a result of expansion of the otters' range.

Finally, the potential for an oil spill must be mentioned in any discussion of public programs aimed at protecting the sea otter because this issue is responsible for the southern population's being listed under the Endangered Species Act. To date, there has been no real conflict with the oil industry in this regard (Tinney, 1983). However, a related set of costs to society has to do with a public mandate to translocate a portion of the otter population to a different area in order to assure that some animals would survive in the event of a localized offshore oil spill. These costs have been addressed in a Preliminary Draft Environmental Impact Statement recently completed by the U.S. Fish and Wildlife Service, a study on which I served as an expert reviewer this year, incorporating many of my own project results.

This project addressed two primary benefits: existence values and tourism values attributable to the presence of the California sea otter population. In Table 3, results of a contingent valuation survey are presented, along with the socio-economic characteristics of the average respondent. The survey was conducted over a random sample of 1000 California households where a response rate of 20% was achieved. The WTP value, or willingness-to-pay estimate, reflects the average value reported by respondents as the measure the household would willingly contribute to preserve the sea otter population. Econometric techniques were utilized to eliminate likely outliers from the response set, and the average values for socio-

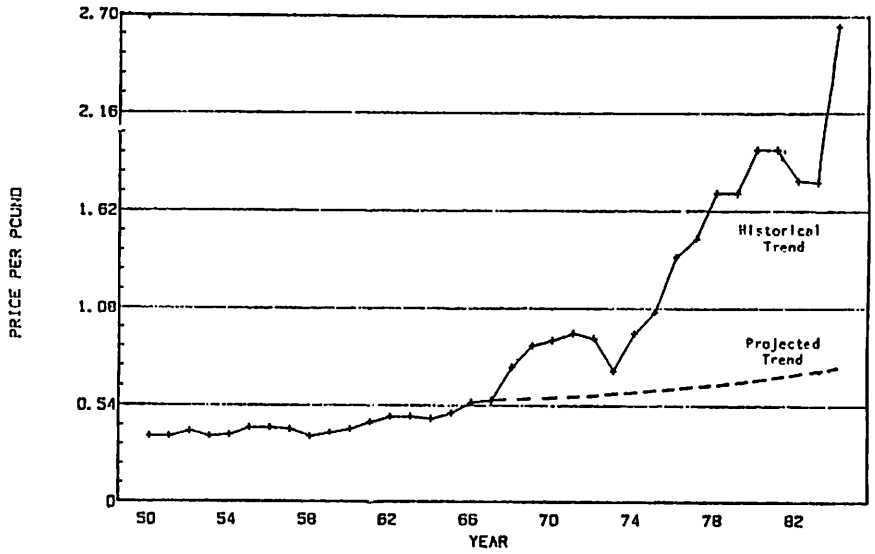

Figure 2. Abalone price per pound, Santa Barbara area (1984 dollars).

Table 2. Sea Urchin Landings (1984 Dollars)

Los Angeles Ports

Year	Pounds (Thousands)	Price/lb	Value ($000)
1971	0	$ .00	0
1972	0	.00	0
1973	137	.18	25
1974	71	.14	10
1975	220	.14	32
1976	143	.17	24
1977	1,669	.16	273
1978	2,686	.17	468
1979	4,579	.19	878
1980	2,859	.20	566
1981	4,659	.20	918
1982	3,104	.18	549
1983	2,906	.22	629
1984	2,674	.24	652

Santa Barbara Ports

Year	Pounds (Thousands)	Price/lb.	Value ($000)
1971	0.2	$ .46	.09
1972	72	.14	9
1973	3,444	.17	601
1974	6,893	.14	980
1975	7,195	.14	1,031
1976	10,472	.16	1,674
1977	13,065	.16	2,073
1978	10,095	.17	1,761
1979	19,822	.15	2,888
1980	17,013	.20	3,385
1981	19,221	.21	4,113
1982	13,367	.19	2,513
1983	11,069	.21	2,312
1984	11,254	.24	2,703

Source: California Department of Fish and Game

economic characteristics were found to be very similar to those of the average California household. Also an independent sample of results on reported avidity levels (ranked on a 0-to-10 scale) for marine recreation (AVIDITY 1) and for wildlife/wilderness preservation (AVIDITY 2) did not show that avidity levels of respondents in the sea otter study were greater than general avidity levels among Californians.

A preliminary contingent valuation study on tourism attributable to the presence of sea otters in the Monterey and Morro Bay areas is presently underway. Data are being compiled on the computer and will be analyzed to ascertain if a willingness-to-pay value exists for on-site observation of the otters in North-Central California. However, it should be noted that the results of the existence value study described above indicate that a large portion (approximately 70%) of the WTP estimate reported was not tied to any actual "use" in the form of on-site observation.

Lastly, another benefit of the sea otters' comeback in California exists. This takes the form of ecosystem linkages. Because the otter has begun to reclaim its range (before fur trading), shellfish populations have probably been reduced to historical levels and, as a result, evidence has been presented (Van Blaricom, 1985) that kelp forests have made a comeback as well (due to reduced herbivore pressure). These nearshore ecosystem impacts are very long-term and not easily identified given the existence of other concurrent environmental effects; furthermore, since only one firm harvests and processes kelp on the West coast, disclosure of economic data on the value of kelp in this area is not forthcoming. Therefore, though we are pursuing a variety of routes in order to make a rough estimate of the incremental value of kelp production resulting from the presence of the southern sea otter, the results on this aspect of benefits are uncertain.

The final stages of the project will be devoted to pulling together those aspects of benefits and costs relating to protection of the southern sea otter which have been quantified during the past year's efforts. Of primary importance will be the presentation of the nonmarket benefit valuations, since this study is one of the first to look at existence values for nonharvested wildlife. The final report, to be compiled in the beginning of 1986, will provide a summary of economic values from a variety of sources (e.g., U.S. Fish and Wildlife, 1985) so that interested citizens and industry groups, as well as government managers, can make overall comparisons of relevant benefits and costs. In an era of heightened fiscal accountability in public programs, both the calculations and methodology used in this study for determining society's valuations associated with marine management choices can provide a basis for making a variety of policy decisions.

Table 3. Willingness-To-Pay for Preservation and Socioeconomic Characteristics (Average/Household)

	Mean Values (Standard Deviations)
WTP/year	$20.75 (25.56)
Seen otters on film	63%
Seen otters at oceanaria	53%
Seen wild otters	25%
Miles to coast	22.9 mi.
Family size	2.7 persons
Age of respondent	42.3 years
Household income	$34,994/year (22,739)
Education	15.4 years (2.9)
Hunt/fish	37%
Member of environmental organization	18%
AVIDITY1 (0 - 10 scale) (marine recreation)	3.4 (3.0)
AVIDITY2 (0 - 10 scale) wildlife/wilderness preservation)	7.3 (2.6)

Cooperating Organizations

California Department of Fish and Game
California Department of Parks and Recreation
National Marine Fisheries Service
U.S. Fish and Wildlife Service

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The California Fisheries and Ocean Policy: State and Federal Dimensions, 1945-85

University of California, Berkeley
R/MA-25
Project Initiated: October 1, 1984
Project Completed: September 30, 1985

Harry N. Scheiber

This project is an investigation of ocean policy-making processes as they have related to the California fisheries since 1945. It seeks to advance public and scholarly understanding of how the ocean sciences and policy initiatives have influenced important developments in the national, regional, and international management of marine fisheries.

During fall 1984, Scheiber analyzed material from the University of Washington Manuscript Collections and the University of Washington Archives, including the papers of leading marine fisheries scientists active since 1945 and the papers of two major leaders in the salmon industry. Additional research has been conducted in the San Diego State University Library, the Scripps Institution of Oceanography Archives, the Hoover Institution Archives, the National Archives (Washington, D.C.), the Princeton University Manuscripts Library, the University of Hawaii Library, and the Hawaii State Archives.

In addition to the foregoing search for and analysis of materials by Scheiber, the trainee located and copied an extensive body of materials in the National Archives bearing on international aspects of Pacific marine fisheries science and management and on related matters of policy and marine research. In addition, Scheiber and the trainee have studied the entire files of the journal *Pacific Fisherman*, and all Congressional hearings of the period relevant to California fisheries, the 1952 International North Pacific Fisheries Convention, and related issues of research policy and management policy and implementation for the Pacific region. All Oregon and Washington state policies were also studied, for purposes of developing a comprehensive view of West Coast developments.

In sum, an exhaustive examination of West Coast, insular, national, and diplomatic archival sources has been completed. This forms the data base for a comprehensive study of California fisheries developments from 1945 to 1985 as they have related to national and international policies and programs, developments in Law of the Sea, and overall economic change in the Pacific Basin region.

These investigations document well a series of issues that will be treated in monographs and in a book-length study. These issues include the following:

1. *The accommodation of diverse regional interests of the Atlantic, Gulf, and Pacific marine fisheries in both national policy formation and the institutional structures of the federal government and international research and management agencies*: Analysis is already substantially completed on (a) the 1945 Truman Proclamation on High Seas Fisheries, its background, impact, and modification by U.S. policy makers; (b) the organization and early contributions of the Pacific Ocean Fishery Investigations project (U.S. Fish and Wildlife Service) based in Hawaii; and (c) the overall development of research and putative management regimes in the Pacific Basin. (McEvoy and Scheiber, 1984, dealt in detail with one segment of the emergent system of regimes for the sardine; others will be treated in the projected book and in Scheiber, 1986.)

A major contribution will be to show how the postwar period, far from being one of relative inaction with respect to ocean science (as argued by Abel, 1981, p. 6), was in fact a period of vital ferment both in oceanography and in policy development. Previous understanding of U.S. policy on ocean law, research, and fisheries management has rested on excellent studies of national-level developments (Friedheim, 1979; Cicin-Sain, 1982; Wenk, 1974). This research, with its focus on California and the Pacific fisheries, will appraise the interrelated roles in policy of the industry, the scientific community, the state and regional agencies, the national government, and such newly created international agencies as the inter-American Tropical Tuna Commission. This study suggests the need to recognize a much more complicated, "denser" institutional complex and dynamic than has formerly been recognized in the history of postwar ocean policy and fisheries development.

2. *The role of marine scientists and the "new oceanography" in the design and implementation of new management and research programs*: Previous works touching this subject have largely been concerned with Woods Hole and the Atlantic and Gulf regions of research (e.g., Schlee, 1973). Some of the rich archival information collected on this theme since 1982 has been incorporated by McEvoy in his book (McEvoy, 1986) and in Scheiber's study of Pacific Oceanic Fishery Investigations (POFI) (Scheiber, 1986). Interviewing of some key individuals involved in California policy formation and scientific advising processes—especially with regard to the Governor's Advisory Commission on Ocean Resources (GACOR) and Advisory Commission on Marine and Coastal Resources

(CMC) studies—remains to be done before this phase of the project can be completed. The Papers of Wilbert Chapman, Milner Schaefer, Roger Revelle, and others have been studied for information bearing on California scientific advising and policy formation.

3. The political economy and diplomacy of Pacific Basin fisheries: A large theme that has emerged in the course of the year's research is the political economy and diplomacy of the commercial fisheries of the Pacific Basin at large. This involves issues such as (a) how fisheries resource management (and research on the resources) has related to changing markets for ocean food products, nationally and internationally; (b) how the "large design" of U.S. foreign economic policies have influenced the West Coast commercial fisheries, especially vis-à-vis Japanese competition and rivalries; (c) and how what this study will term "corporativism" (direct involvement of industry leaders and scientists in the policy process) and its role in national and international relationships have affected the scientific community, industry, and management agencies. A start has been made toward tracing a highly important set of developments—the relations of the United States and Japan with respect to Pacific fisheries—for which even the rudiments of narrative and analysis are absent in the literature (e.g., in Borden, 1984, which is the latest study).

Scheiber has presented some of the research findings already, especially in major presentations to the International Congress of History Sciences, August 1985 (at Berkeley) and the All-University of California Economic History Conference, November 1985 (at Asilomar), and also in lecture presentations at Princeton University (May 1985) and New York University (October 1984). Scheiber has also served as adviser to the Roger Revelle Oral History project (conducted by his former doctoral student, Sara Sharp), shared research materials extensively for inclusion in McEvoy's major book on California fisheries law and policy (McEvoy, 1986), and advanced considerably the work of bringing research findings from this project into book manuscript form for completion sometime in 1986. Additionally, materials from the project have been used in development of a unique new course at Boalt Hall on "Law, Resources, and Technology." Finally, early completion of a small working paper or monograph on scientific advising and ocean policy development in California, with a trainee as coauthor, is expected.

Cooperating Organizations

Hawaii State Archives
Hoover Institution
Princeton University
San Diego State University Library
University of California, San Diego, Scripps Institution of Oceanography Archives
University of California, Berkeley, California Academy of Sciences
University of Hawaii
University of Tokyo, Department of International Relations
University of Washington
U.S. National Archives, Washington, D.C. and Suitland, Maryland

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Scientific Liaison, Joint Research Planning, and Information Exchange Among Alta California, Baja California, and Gulf of California Marine Scientists

University of California, San Diego
A/S-1
Project Initiated: October 13, 1983
Project Completed: September 30, 1985

George T. Hemingway

Introduction

The Interamericas Program at the Scripps Institution of Oceanography has, as its purpose, the provision of liaison, coordination, and facilitation functions between U.S. and Latin American marine scientists. The U.S.–Mexican portion of these functions has been supported through the assistance of Sea Grant International Program funds and, until September 1985, Sea Grant College Program funds. The non-Mexican portion of this program has been funded through other private funds. Both increments are sustained by a substantial contribution of volunteer service, in-kind facilities and services, ships and other carriers of opportunity, and good will. Evidence of Scripps Institution activities in support of Mexican marine science development extends back to at least as early as 1960. A formal program was established in 1978, based on existing successful relationships and activities.

The longest running successful activities of this program and its antecedents are bibliographic assistance and short courses, lectures, and conferences.

Bibliographic Assistance

The Program Office advertises among the academic communities of southern California for donations of journals, textbooks, references, and reprints. Most of these donations come from individual researchers who need more space in their offices or who are retiring from active service. Some very large and valuable collections have come from academic libraries that are consolidating holdings or closing out holdings. Shipment to San Diego is normally managed by the donor. Within the greater San Diego area, volunteers retrieve donated materials for sorting in our redistribution center. These donated bibliographic materials reach their final destinations in Latin American marine science libraries through a diversity of mechanisms. Seldom are materials shipped by commercial carrier unless gratis shipping can be arranged with the carrier. Foreign naval vessels have transported materials to ports near to the receiving institution. NOAA and SIO vessels have transported materials when they are fueling at ports near to client institutions. Nearby laboratories send trucks to La Jolla, and their staffs assist in sorting and selecting materials and taking them to their home institutions. In this manner, about 500 boxes of bibliographic material have arrived at client libraries during the past year.

When funding is available, the Program will respond to requests for computer bibliographic searches for client laboratories. Some clients have established accounts with us for those services.

Courses, Lectures and Conferences

During the past year the Program has coordinated and facilitated attendance of U.S. and Mexican marine scientists at several conferences including the following: the annual CalCOFI (California Cooperative Oceanic Fisheries Investigations) Conference, where researchers from California, Washington, Oregon, Mexico, Peru, Chile, Argentina, and Canada presented papers (these conferences are both aided by simultaneous English–Spanish and Spanish–English translation); the SARP (Sardine/Anchovy Research Program) training sessions sponsored by IOC and NOAA, where fisheries researchers from Argentina, Chile, Peru, Ecuador, Mexico, Spain, Brazil, and Norway joined with their U.S. colleagues in preparing for a major worldwide initiative in fisheries research.

Liaison Functions

During the last 5 years, CICESE (Centro de Investigaciones Cientificas y Educacion Superior de Ensenada), a Mexican federal research laboratory, has initiated a major research thrust in the Gulf of California. Multidisciplinary (physics, chemistry, biology, and meteorology) cruises on Mexican and U.S. vessels have vastly increased understanding of the dynamics of the Gulf. Cooperative ventures involving SIO, CICESE, U.S. Navy, the Mexican Navy, University of Mexico, Mexican Fisheries personnel, and vessels of the U.S. Navy and Mexican Navy, the University of Mexico, and SIO, have made these studies possible during times of budgetary stress at both U.S. and Mexican institutions.

In the past year 5 Mexican scientists and students have participated in CalCOFI cruises.

The Interamericas Program Office coordinated participation of shipboard personnel in these cruises and dealt with precruise planning, coordination, and communication problems.

The Program Office provides a source of information about current conditions in Mexican laboratories, updated telephone numbers and addresses, and bilingual services in

communicating with Mexican and other Latin American colleagues and with government officials. These services are valued by persons attempting to perform joint research, to obtain data, to obtain ship operations clearances and collecting permits, to obtain information about officially assigned observers, or simply to travel to meetings. A combined incoming and outgoing foreign telephone call load of near 800 calls per year (an average of about 3+ per day) attests to the need this program meets.

Future Plans

Both Sea Grant and private grant funding for this program have terminated. Certain facets of the program continue; others have been terminated or drastically curtailed. We continue to solicit and receive books, journals, and reprints. Mexican librarians continue to come and review our holdings and take what they need. Volunteers continue to sort donated materials and catalogue them, as well as provide local pick-up service. We still look for ships and carriers of opportunity to deliver materials to other, more distant users. We continue to provide such information as we have on the status of foreign laboratories and their phone numbers and addresses. We continue to attempt to put U.S. and Latin American colleagues in touch with one another.

We are no longer be able to provide translation services, telephone communications, or rapid response to situations that we have provided in the past. Written communications are also extremely curtailed and most communications must be in English, because a dedicated secretary for these functions is no longer available.

Four thousand dollars toward the establishment of an endowed scholarship for Latin American graduate students to study at SIO has been received. About $150,000 will be needed to fully endow the scholarship fund.

Cooperating Organizations

Centro de Investigaciones Cientificas (CIB)
Centro de Investigaciones Cientificas y Educacion Superior de Ensenada (CICESE)
Centro Interdisciplinario de Ciencias Marinas (CICIMAR)
Instituto Nacional de la Pesca (INP)
Mexican Naval Oceanographic Laboratory at Manzanillo
Mexican Naval Vessel *Matamoros*
People-to-People Program, University of California, San Diego
R/V *David Starr Jordan*, National Marine Fisheries Service, National Oceanographic and Atmospheric Administration
R/V *New Horizon*, University of California, San Diego
San Diego State University
Universidad Autonoma de Baja California (UABC)
Universidad Autonoma de Baja California Sur (UABCS)
Universidad Nacional Autonoma de Mexico (UNAM)

Rapid Response

BLanK

Domestication and Genetic Improvements of *Sargassum* species

University of California, Santa Barbara
R/NP-1-14A
Project Initiated: October 1, 1984
Project Completed: September 30, 1985

Aharon Gibor

During the past 20 years, plant tissue culture has been used successfully to enhance the commercial value of terrestrial plants (Murashigi, 1977). Marine algae have many uses throughout the world (Chapman, 1970; Misawa, 1977). During the last 10 years research has increased in the area of algal tissue culture (Field and Lovlie, 1976; Saga et al., 1978; Van Der Meer, 1979; Gibor et al., 1980; Polne et al., 1980). Our lab has pioneered the development of techniques for algal tissue culture. During the last 12 months we have concentrated our research on *Sargassum muticum*, which grows locally and is rapidly becoming abundant on the California coast. Seaweed has been suggested as a possible fuel alternative. This renewable resource can be harvested and fermented to produce methane (North et al., 1982; Shelef and Soader, 1980). Selection, mutagenesis, and fusion experiments will make it possible to select the fastest growing individuals which can yield the highest fermentable biomass and to develop strains which will have optimal growth at different temperatures and light regimes.

The major goals of our project this year were sixfold. The first goal was to look in depth at our cleaning techniques. Tissue culture requires axenic material, especially when using nutrient-rich media. Our standard procedure was as follows. First, pieces of algae were brushed under a dissecting microscope to remove epiphytes and larger particulate matter. Second, these pieces were sonicated in sterile seawater for further removal of contaminants. Third, the sonicated material was treated with "Betadine" solutions. Fourth, the treated pieces of algae were suspended in double distilled water for short periods to aid in the removal of microorganisms without cell walls. Finally, the pieces were treated in an antibiotic mixture. Each of these treatments was examined in combination and separately after a short resting period of usually 1 day. Pieces of tissue were placed onto slides and checked with fluorescent microscopy. Sonicated tissue, as well as that suspended in distilled water, showed adverse effects in a number of cases. We also checked the use of alcohol and sodium hypochlorite (common cleaning agents in terrestrial plant tissue culture), but found these to be both detrimental to the cells and not effective in preventing contamination. Identical pieces of tissue that were examined with fluorescent microscopy were also plated on nutrient-rich agar. These sterility tests and fluorescent microscopy findings resulted in a change in the general cleaning procedure. Both sonication and distilled water treatments have been omitted from the cleaning procedure without affecting the production of axenic tissue to be used for further experiments.

The second goal of our project was to determine the best way to store collected live tissues. Tissue can be stored in three ways: in aquariums in the lab, in seawater tanks outdoors, or in the refrigerator. Aquarium storage in the lab has proven to be the least efficient. Contamination problems increase, although the tissue does remain viable. Storage outdoors also results in additional contamination problems, but is preferred to the latter. Tissue which is moist and wrapped in the refrigerator for up to 1 month is the most convenient method. This method of cold, dark storage may also prove to be helpful in increasing our production of protoplasts, although this must yet be documented. Storage of tissue is necessary when working with species which are not locally available or which are available during a short season only.

The third part of our research has been to focus on a reliable method for obtaining viable protoplasts from *Sargassum muticum*. Two enzyme sources were tested. The first was enzymes extracted from the gut and hepatopancreas of abalone and from sea urchins. While these enzymes proved to be active, at times their activity was not always repeatable. The second source was commercially available enzymes. Thirty different enzymes were tested separately and in combination. After we tried various ways of preparing the enzymes, a set procedure was determined. Enzymes were dissolved in cold sterile seawater and were stirred overnight in a cold room. The enzyme mixture was then centrifuged at 15,000 rpm in an ultracentrifuge for 40 minutes. The supernatant was then filter sterilized and divided into small aliquots and frozen until needed for use. A wide variety of buffers and osmotic stabilizers was tested. Sorbitol has become a necessary component of the enzyme solution, which also contains cellulose "Onozuka" RS and limpet acetone powder. Small pieces of clean tissue were placed in the above mixture and gently spun on a rotor wheel overnight. The tissue was then soft enough to be squashed on a slide or gently ground with a loose-fitting tissue grinder; it then released many healthy pigmented and slightly pigmented protoplasts. Released along with the protoplasts, however, were many broken cells, cell walls, chloroplasts, and pieces of tissue.

Our fifth goal, therefore, is to try to

find a better way to disassociate the softened tissue or remove these contaminants. Our goal is to produce large numbers of protoplasts exclusively. At this time we have tried sucrose, dextran, and Ficol gradients with only moderate success. The same is true for filtering and centrifugation methods. We have been able to cut down on the number of these contaminants and feel this obstacle will be overcome shortly.

As protoplasts are produced, we have plated them on various agar solidified and liquid media. These include Van Stochs media; $ASP_{12}NTA$; Provasoli-enriched seawater (PESI) plus iodine; various sterility test media; and PESI plus antibiotics, sucrose, yeast extract, glucose, and numerous plant hormones. At this time we have not found a medium which is effective for growth and regeneration.

Protoplast viability has been shown by use of fluorescent microscopy and vital stains such as Evans Blue and Neutral Red. The absence of cell walls has clearly been documented with calcifluor white and the fluorescent microscope.

Cooperating Organizations

Gas Research Institute, Chicago
University of Florida, Gainesville

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Relationships Among Ploidy, Success of Eyed-Larval Settlement and Early Growth in the Pacific Oyster

University of California, Davis
Bodega Marine Laboratory
R/NP-1-14B
Project Initiated: October 1, 1984
Project Completed: September 30, 1985

Dennis Hedgecock

California's oyster industry is based upon extensive culture of the Pacific oyster (*Crassostrea gigas*), an introduced species that does not reproduce locally. In the 1980's this industry became dependent upon seed produced by hatcheries and shipped to growers as eyed-larvae for on-site setting (Chew 1984). Short-term problems with this method (poor on-site setting success and lack of control over seed quality) as well as long-term opportunities for genetic improvement through controlled hatchery reproduction (Hershberger et al. 1984) were addressed by this project. In particular, we sought to improve utilization of imported oyster seed by (1) investigating conditions promoting success of eyed-larval metamorphosis and (2) examining the survival and early juvenile growth of triploid eyed-larvae. Commercial hatcheries and Sea Grant researchers in Washington are studying triploidy as a means of producing sterile oysters for marketing during the normal spawning season (Allen et al. 1985). To achieve controlled hatchery reproduction of Pacific oysters we proposed also (3) to study seasonal variation in the gonadal condition of local stocks and in our ability to condition and spawn broodstock throughout the year.

Reproductive Patterns of Adult Pacific Oysters

Samples of mature oysters were obtained in almost all months from commercial oyster beds in Tomales Bay (two sites) and Drake's Estero. Tissue samples were taken for histological study from five individuals of each sex for each locality in the months of November, December, January, February, and April. Seasonal patterns of gonadal development in these samples generally follow those described previously by Berg (1969). In order to assess our ability to condition and spawn animals throughout the year, adult oysters were collected from these same localities on a bimonthly schedule beginning in mid-November. Broodstock taken directly from the field were spawnable from May through August, but spawning and fertilization were achieved from January through October by advancing (Muranaka and Lannan, 1984) or retarding gonadal development through appropriate temperature manipulation.

Causes of Larval Mortality in the Hatchery

Survival of larvae produced in early experimental crosses, however, was highly variable and in general quite poor. Initially 9-liter plastic pails were used for larval culture since, in contrast to the massive scales of larval rearing in commercial hatcheries, our experiments require the rearing of many family groups with relatively few oysters per family. Inability to rear sufficient numbers of larvae to metamorphosis consistently in these pails, however, forced us to examine experimentally the causes of larval mortality. Although this represented a departure from our stated goals, we felt that these studies were necessary if we were to carry out our objectives of studying metamorphosis and larval settlement, and that the results would be relevant not only to the widespread problem of unpredictable mortalities experienced by commercial hatcheries (see Lannan, 1980) but also to other bivalve genetics programs faced with the constraints of replicating larval rearing units for family studies (S. Allen, Univ. of Washington, pers. comm.).

Alternative hypotheses to explain observed larval mortalities were tested in five major factorial experiments. Unless otherwise noted, hatchery methods generally followed those of Breese and Malouf (1975) with Tahitian-*Isochrysis* as the algal diet. Abrupt declines in larval numbers within the first week of culture at first suggested a toxicity problem. Because volatile toxic substances may be imparted to larval cultures aerated by mechanical pumping (Breese and Malouf, 1975), the first experiment compared two sources of air (the "house" supply from a large-volume, low-pressure Schram pump vs. a Silent Giant aquarium air pump), two volumes of aeration, and two bubble sources (airstone vs. glass rod) in a full 2 x 2 x 2 factorial experiment. Each treatment was replicated in three 9-liter pails initially stocked with 50 fertilized eggs per ml from a mass spawning of three males and two females. ANOVA of the number of larvae per ml on day 3 revealed that house air and low air volume were significantly the best treatments. Nevertheless, by day 7 few larvae were left in any pail. Aeration was again tested as part of a second experiment in which activated charcoal-filtered vs. unfiltered house air were compared and found to be not significantly different. Finally, as part of a fourth experiment, low vs. high air volume were again compared. Although the day 3 survivals were not different, by day 10 population crashes had occurred in all but five cultures which were on low air volume. Better survival of cultures on low aeration is yet unexplained, but seems not to be the result of toxicity

of the air supply.

Properties of the culture vessel were next examined under the hypothesis that the material or size of the container was a significant factor in mortality. As part of our second experiment, larval survival rates were compared for four vessels of different diameter, 4-liter plastic cylinders, 4-liter glass beakers, 9-liter pails, and 17-liter buckets, each containing only 4 liters of culture water. ANOVA of day 3 survival showed cylinders > beakers > pails > buckets, suggesting that the *height* of the water column, not the vessel's material, was a significant factor in survival. These and other results suggested that 17-liter buckets (filled) do provide sufficient survival for rearing family groups. In a third experiment, therefore, we varied height and volume together by comparing two replicates each of four container sizes/shapes relative to standard 17-liter buckets as follows: 113-liter, 48-cm-diameter plastic trash cans filled to a depth of either one (30 cm) or two buckets, and 14-liter 15-cm-diameter PVC cylinders filled either one or two buckets high. Results suggest that larval survival was more sensitive to height of the water column than to surface area, i.e., a doubling of height has a greater effect upon survival than an order of magnitude difference in surface area (Figure 1).

In experiment two, by day 10, five beakers, two pails, and one cylinder that had had densities of 5 to 20 larvae/ml at day 3 were found to have less than 0.5 larva/ml, suggesting that population crashes may have been due to initial densities being too high. The density effect appeared to be separate from the water column height effect noted above since day 10 survival was highly correlated with day 3 survival for those containers that did not crash (r = 0.755, 10 degrees of freedom, $p <$ 0.0025). A fourth replicated factorial experiment was therefore designed to test the effects of three larval densities (10, 30, and 50/ml) at three densities of algal food (3, 6, and 12 x 10^4 cells/ml) in 17-liter buckets. Algal density had no effect on larval survival, but larval density did. Day 6 survivorship was significantly higher in the 30/ml than in the 50/ml larval densities (F = 6.09, $p < 0.05$); survivorship at the lowest density was comparable to the 30/ml density, but total counts were of course lower. By day 10 crashes had occurred in all but five buckets that were initially at medium or high densities. These, however, retained on average only 1.2 larvae/ml, a survivorship of about 3%.

Having eliminated toxicity of air supply or culture vessels as explanations of such poor survival, we did a fifth replicated factorial experiment to test sanitation and handling stress as possible causes of mortality: two methods of bucket cleaning (scrubbed vs. scrubbed *and* bleached) x two frequencies of culture water replacement (2 day vs. 4 day) x two screening techniques (culture poured directly through Nytex screen vs. pouring onto a screen submerged in a dishpan). By the second week of this experiment,

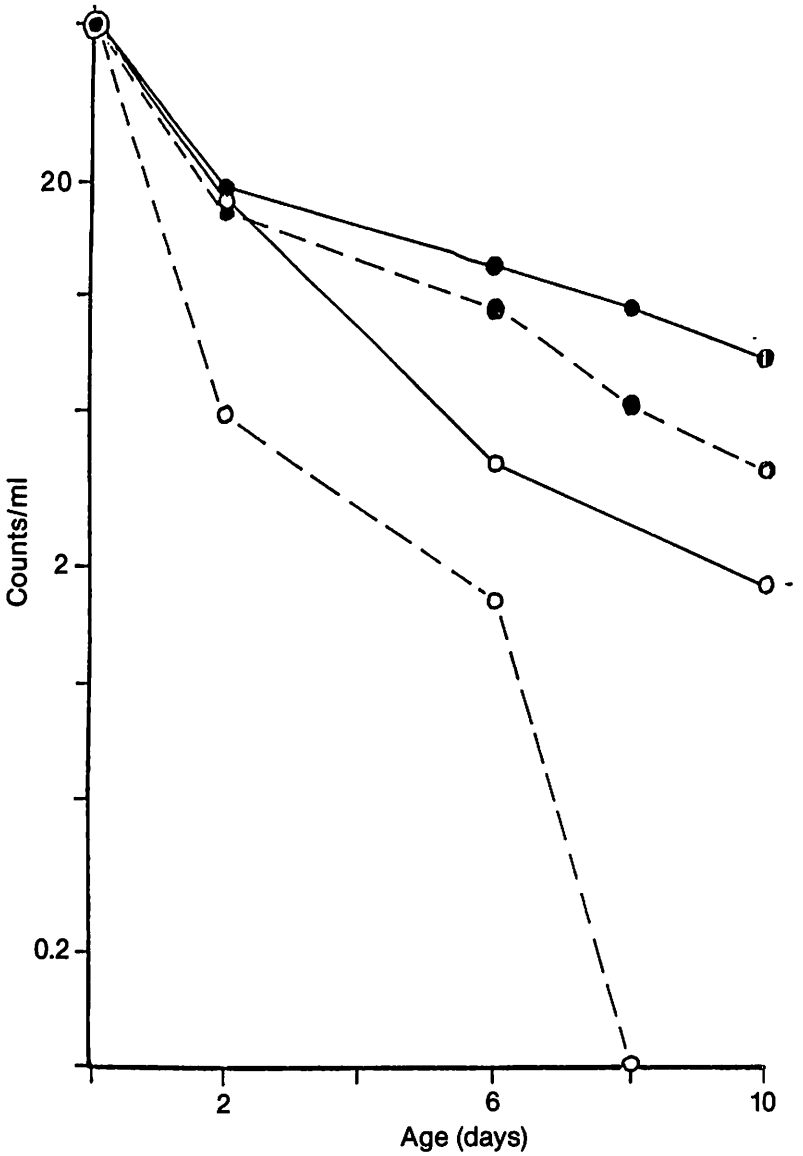

Figure 1. Survival in larval culture containers of different configurations, initially stocked at 50/ml. Solid circles, water column 60 cm high; open circles, 30 cm high; solid lines, 1,800 cm^2 surface area; dashed lines, 180 cm^2 surface area.

cultures on two-day water replacement schedules had significantly higher survival than those on four-day schedules ($p = 0.0001$), roughly screened animals had survived better than gently screened ones ($p < 0.01$), and bleaching had no effect (Figure 2). Days 7 to 9 appeared to be a critical period for crashes.

We are now designing tests of two alternative hypotheses to account for these results: (1) microbial fouling of surfaces and larvae is a significant cause of mortality or (2) water quality (ammonia level?) fluctuations cause poor survival.

Factors Affecting Success of Metamorphosis and Settlement

A two-factor (3 x 3) experiment tested the effects on settlement of two chemical inducers of metamorphosis (L-dopa and epinephrine at 10^{-4} *M* vs. seawater control) (Coon et al. 1985) and of larval feeding in the last day before metamorphosis (starved vs. 1x and 2x normal algal cell density). The left, cupped valves of Pacific oysters were used as the experimental units, five per treatment. Eyed-larvae were pipeted into the shells, counted, treated for 1 hour, and then decanted; the number of spat remaining attached was then counted. Striking and contrasting effects were seen for the chemical inducers (Table 1); as reported by Coon et al., L-dopa promotes metamorphosis with a high percentage of attachment while epinephrine promotes metamorphosis (not tested here) without attachment (with significantly lower set than control). Larval diet also significantly affected the percentage of spat set; fewer starved than fed larvae set in each case and overfed larvae did better than normally fed larvae only in seawater (Table 1).

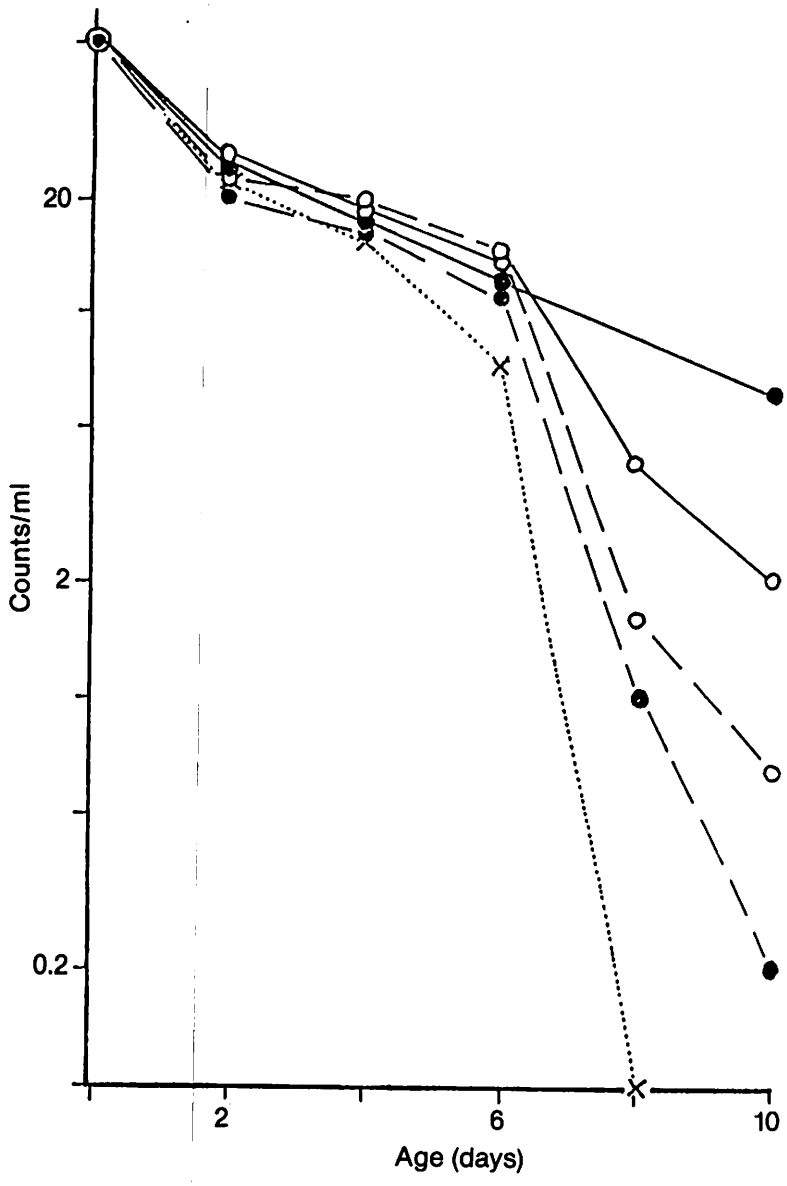

Figure 2. Survival of larval cultures under different conditions of handling, initially stocked at 50/ml. Solid circles, cultures roughly screened; open circles gently screened; solid lines, cultures screened and water changed every two days; dashed lines, water changed every four days; dotted lines with x's, cultures run in parallel with the factorial experiment three with no replacement of water.

Production of Triploid Spat

After several unsuccessful attempts to induce triploidy using the cytochalasin B method of Stanley et al. (1981), we finally produced spat in September. Eggs from a mass spawning were treated with 0.1% CB in DMSO from 40 to 55 min. postfertilization to inhibit formation of the second polar body. From a 113-liter trash can initially stocked with 50 larvae/ml, we obtained less than 10^3 treated spat, a survivorship of only about 0.001%, which is comparable to Allen et al.'s (1985) results. Survivors are being reared in a newly constructed upwelling nursery system to sizes suitable for planting in the field.

Cooperating Organizations

Washington Sea Grant College Program, University of Washington, Seattle

Coast Oyster Company, Quilcene, Washington

Great American Shellfish Company, Marshall, California

Hog Island Shellfish Company, Marshall, California

Johnson's Oyster Company, Inverness, California

Tomales Bay Oyster Company, Point Reyes Station, California

Table 1. Number of Pacific Oyster Eyed-Larvae Used and Spat Set (Summed Over Replicates) in an Experiment Testing the Effects of Chemical Inducers of Metamorphosis and of Larval Feeding

	Amount of Algae Fed 1 Day Prior to Set					
	None		1 x Normal		2 x Normal	
Inducers	Larvae	Spat	Larvae	Spat	Larvae	Spat
Seawater Control	86	9	114	13	61	16
L-Dopa	61	16	148	49	90	29
Epinephrine	63	1	77	4	52	2

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New Challenges Ahead in Managing Santa Barbara Channel Resources

University of California, Santa Barbara
R/NP-1-14F
Project Initiated: January 1, 1985
Project Completed: September 30, 1985

Biliana Cicin-Sain

The primary goal of the project was to provide a synthetic characterization of the potential implications of the recent major acceleration in offshore oil activity in the Santa Barbara Channel for other users of the marine environment and for cumulative regional impacts. Offshore oil activity is projected to increase dramatically in the Santa Barbara Channel. Fourteen major separate oil development proposals (calling for an ultimate production level of 500,000 to 800,000 more barrels of oil per day by 1992) are being considered for approval or denial by local, state, and federal authorities. It is clear that the magnitude of the development that is proposed and the rapid succession of proposals that need to be analyzed (each on its separate regulatory "time clock") is taxing the capacity of government agencies, of representatives of marine users, and of the general public to fully comprehend the cumulative regional impacts and specific impacts on other marine users that may take place. The purpose of this project was to provide independent outside review of the processes whereby cumulative impacts and impacts on other users are ascertained to assist public agencies and private sector users of the channel. Similarly, it was expected that an overview account of the multiple use issues faced in the Santa Barbara Channel region would provide useful information to national level decision makers in the Congress and the executive branch as they prepare to consider reauthorization of and amendments to several key ocean laws.

Challenges to Government and Public Interest

Two major papers have been prepared under the project. The first is entitled "Offshore Oil Development in California: Challenges to Governments and to the Public Interest," and it characterizes the major challenges to governments and to the public that are posed by the accelerated pace in oil development which is now taking place offshore California. The first part of the article characterizes the magnitude of the current oil build-up, placing it in historical perspective. Next, the major issues posed by accelerated development are described, as is the complex of federal, state, and local laws that govern offshore oil extraction. Problems in this regulatory framework are then analyzed, and management dilemmas faced by local authorities in coping with the increase in oil activity are highlighted. The flow of government revenues derived from oil and gas development at both federal and state levels is analyzed next. The article concludes with a discussion of the need for change in the way the decision-making process is structured, in the flow of federal and state oil revenues, and in patterns of citizen involvement. These conclusions are summarized below.

Reforming the Decision Process. A variety of avenues for necessary reform flow from the analysis of the governance problems discussed in the paper. There are several prominent problems at the *federal* level. First, Congress has to better harmonize the body of laws that affect ocean resources and uses, and provide for public mechanisms for conflict resolution when these laws conflict with one another. Second, several amendments to the Outer Continental Shelf (OCS) Lands Act Amendments should be considered: (a) to incorporate more fully state and local input into the oil-leasing process; (b) to provide more specific standards to assist the Secretary of Interior in balancing national energy needs with adverse impacts on other resources and uses; and (c) for ocean areas already leased, to require the Secretary of Interior to set offshore air pollution standards consistent with air pollution standards onshore. Third, Congress needs to clarify the meaning of "consistency" under the federal Coastal Zone Management Act.

At the *state* level, there is a need to consider altering the Permit Streamlining Act (PSA) to allow additional time for environmental review of the megaprojects being proposed for offshore California (amendments to the PSA are already under consideration). In light of the projected massive development offshore, the further erosion of the California Coastal Commission—in terms of budget and staff—needs to be halted so that the agency can continue to fulfill its legal obligations and participate in the necessary intergovernmental review processes.

At the *local* level, local officials must make the time and get the money needed to get away from the project-by-project approach and begin to do much needed long-range, comprehensive planning. Planning efforts at the Santa Barbara county level, should also be coordinated with those of the two other counties affected, Ventura and San Luis Obispo, because the development and the socioeconomic consequences that are likely to accompany it are very much regional in scope.

Changing the Money Flow. If the states and localities are to be able to do the advance planning that is needed and cope with the adverse impacts that may ensue, the flow of

revenues from offshore oil development must change. A portion of the federal revenues must be diverted back to the affected states and localities, and a greater portion of state oil revenues must be redirected toward the impacted localities. Given very legitimate concerns with the growing federal deficit, the chances for enactment of some type of ocean revenue sharing bill may be enhanced if the scope of existing OCS revenue sharing efforts were broadened to include sharing of revenues from future ocean uses such as ocean mining. Moreover, the formula could be tied to both the level of *new* (rather than existing) activity and revenues involved, as well as to the distance of the development to shore (e.g., the greater the distance, the fewer the revenue benefits, and conversely, the closer to shore, the greater the benefits).

Broadening Citizen Involvement. Finally, citizens must regain control over the oil planning and development process, which is now almost entirely in the hands of the experts, the technocrats. The ultimate guardians of our ocean resources, after all, are the citizens of the United States, who are the ultimate owners of the bountiful resources of the sea. It is public action that must ultimately reflect the public interest in this area. Government agencies cannot be depended on to do the job alone, particularly in the offshore area, because most of the agencies involved have single-purpose mandates very narrowly defined by law. While in the local context of the South Coast, a small number of very able and concerned citizen groups have played a prominent role in the oil planning process, given the nature of the challenges that are posed and the need to influence action at the local, state, and federal levels, a wider public needs to become involved.

It is clearly in the national interest to develop the oil resources offshore California, but it is also in the national interest to develop these resources at a reasonable pace that does not inflict irretrievable damage on other resources or radically alter the economic and social fabric of South Central Coast communities. The derricks that blanketed Summerland in 1896 are no longer there. The area is now once more a beautiful beach. One would hope that this can be done again, even though the current development wave is of a different magnitude than the others that preceded it and the challenges it poses are significantly greater. Meeting these challenges will require more concerted government action at all three levels of government and renewed citizen commitment.

Solutions to Offshore Conflicts

The second paper is entitled "Private Solutions to Conflicts Over Public Resources: How Well Do They Work? Oil/Fishing Conflicts Offshore California." It analyzes the application of mediation to resolve conflicts between commercial fishermen and oil operators in the South Central Coast of California. The first part of the paper provides a capsule history of the case and describes the methodology used in the study which is based on personal interviews with participants involved in the face-to-face oil/fish industry negotiations as well as with other selected members of both industries. The second part of the paper analyzes the interview responses regarding the origin of the conflict, the structure and operation of the negotiation process, the issues that have been addressed in the negotiations, and evaluations of the outcomes of the negotiation process. The final section of the paper provides commentary by the authors on how well this private negotiation effort appears to be working and draws a set of implications for other conflict cases. The respondents' evaluations of how well the process is working are summarized below.

Evaluation of Progress on Issues and of the Negotiations Process. Respondents were asked a set of questions related to the range of issues that had been addressed by the Joint Oil/Fish Committee, their evaluations of the success made in addressing outstanding issues, and general evaluations of the negotiation process as a whole. In general, representatives from both industries were in agreement on which issues had been addressed most successfully by the committee. Respondents agreed that the "easiest" issues, which had been addressed first, had been resolved with a great deal of success. These included the following:

1. The establishment and operation of a Fish/Oil Liaison Office and the much improved communication between the two industries that this office had fostered. Several respondents referred to the very useful role that the office had played in matters such as assisting the fishermen in filing claims with the Fisheries Contingency Fund.
2. The improvement in the system of notification of seismic vessel activity which originally had been one of the major issues in contention.
3. The establishment, on a voluntary basis, of vessel traffic corridors for both industries.

More skepticism was expressed on resource related issues. Although a number of respondents praised the joint fact-finding approach utilized on the fish dispersal and eggs and larvae issues, others also pointed to the slow progress made in this regard and the fact that many years of study will be needed to fully understand the potential impacts of seismic exploration on marine life.

The most skepticism (on the part of both industries, but particularly on the part of the fishermen) was expressed on the issue of compensation for loss of fishing opportunity. A number of the fishermen expressed a general feeling of frustration with the lack of progress on this issue, although they were not willing to discuss the details of the points that remain to be resolved because negotiations on these questions were still very much

in progress. Oil industry representatives also expressed some reluctance on this question, emphasizing their concern with the precedential nature of any agreements on this issue; that is, while fishermen would prefer to institutionalize a compensation scheme, the oil industry would prefer to deal with compensation on a case-by-case basis.

Respondents were also given a more general evaluation question asking for their overall perceptions of this type of conflict resolution approach. Had it been successful, partially successful, or inadequate? Was the private negotiation approach appropriate or inappropriate for the type of conflict in which the industries were involved? On this question, the oil company representatives were uniformly favorable in their responses, while the fishermen and the neutral advisors exhibited mixed responses—some favorable, others unfavorable.

Oil spokesmen consistently reported that the private negotiation approach was "necessary, appropriate, and valuable." These answers stressed the importance of the process itself, i.e., its success in starting conversations and in establishing a more positive relationship between the two industries. Some of these responses, too, underscored the importance of the voluntary approach; as one respondent said, "the voluntary system works well...if government were doing it, we wouldn't have made 1/16 of the progress we have made...."

Fishermen and the neutral advisors, on the other hand, expressed more mixed views. Only one fisherman out of eight responded favorably, saying that it was the "best possible approach under the circumstances" and adding, however, that "fishing was still losing but at least slowing down the losing process." Two other fishermen said that the approach had been partially successful and appropriate (e.g., "some good things have come out of it, but mostly it's the same..."), but also pointed out problems in representation. Five other fishermen expressed serious doubts in their overall evaluations of the process (e.g., "It is just a delay tactic," "The seismic effects on the resource are not being addressed," "I don't think that we should have taken this route, should have stayed in the public arena," "Fishermen should work in the public arena instead," and "If no agreement is reached soon on compensation, the committee should be scratched and the fishermen should go back to the public process"). Other evaluative comments expanded on the theme that the oil companies were only trying to delay the process. One respondent said that the "oil companies are just delaying while they get their permits; they are stalling and delaying, holding meeting after meeting." Another echoed this theme by remarking that "after two years, the oil companies have gotten a lot of mileage out of these meetings...."

Other comments related to the advantages of working in the public, rather than private, realm. One fisherman thought that fishermen should form alliances with the environmental groups and work through public decision makers, otherwise they would never be able to mobilize the needed time and resources to be politically effective. One respondent expressed some fears that the Joint Committee was becoming too powerful; that, in fact, the public agencies were informally beginning to rely too much on the committee for information: "The committee is now making public policy...." A final evaluative comment dealt with the role of the mediators, one fishing spokesman suggesting that the mediators were benefiting themselves.

With regard to the neutral advisors, by and large, they thought that the approach had been partially successful, particularly with reference to the operational issues, but they also expressed some doubts about problems of representation.

We asked our respondents two other evaluation questions: How could the process be improved? and Had it worked to "head-off" or discourage other potential conflicts? On the first question, there was considerable agreement among most respondents that the process could be improved through better communication with the respective industries. Some suggested more frequent meetings, others mentioned industry caucuses, yet others spoke about methods of improving representation and of establishing a rotation system of participation. On the opposite side of the last point, others mentioned that a low turnover in participants was important to establish a level of trust needed for successful negotiations.

On the last question—the extent to which the "getting together" had been useful in preventing other conflicts from arising—an interesting split in opinion is present. The oil industry representatives almost uniformly thought that "just talking helps" and "avoids misunderstandings," that much had been accomplished to contain the problems, and that "without the work of the Joint Committee, things may have been in chaos for both industries." The majority of the fishermen, on the other hand, either simply said "no" when asked this question or suggested that the process had worked to avoid conflicts initially, but that "now it may cause more conflicts because of frustration."

Cooperating Organizations

California Coastal Commission
California Coastal Operators Group
Chevron USA
Minerals Management Service
Oil/Fish Liaison Office, Santa Barbara
Santa Barbara Commercial Fishermen's Association
Santa Barbara County Citizens Planning Association
Santa Barbara County, Energy Division
U.S. House of Representatives, Merchant Marine and Fisheries Committee, Oceanography Subcommittee

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Economic Impact of Recent Changes in the Tuna Industry

ERG, Pacific, Inc.
R/NP-1-14G
Project Initiated: April 1, 1985
Project Completed: August 1, 1985

Dennis M. King

Tuna resources offer unique opportunities for the United States and for other nations because they are international resources that grow to market size in the wild, can be harvested on the high seas, and can be delivered to market without any of the public investments normally associated with shore-based food production. As a form of American enterprise, tuna fishing is unique because most U.S. tuna fishermen engage in long distance search and capture operations in direct competition with fleets from over 20 other nations and then compete with them again to sell the harvest in a highly competitive international market.

The dominant market for tuna is the United States, but many foreign tuna fleets can offer low cost fish to the U.S. market because they are nationalized or heavily subsidized. This makes it difficult for U.S. fishermen to compete even at home. The recent growth of foreign tuna fleets designed to serve the U.S. market and the relocation of U.S. tuna canneries to offshore sites have created special problems for U.S. tuna fishermen, especially those whose operations were designed to support a U.S.-based tuna processing industry. The decline in U.S.-based tuna operations has also generated economic losses in many other segments of the U.S. economy.

The relocation of U.S. processing operations to overseas sites has taken place over many years, but the most recent phase during 1980–84 has been the most dramatic. Although the 157% increase in U.S. canned tuna imports during this period has attracted the most public attention, the economic impacts associated with the relocation of the U.S. tuna operations are more significant. Although the capacity of the U.S. tuna fleet declined by only 14% during this 5-year period, most U.S. caught tuna is being delivered to offshore ports, and U.S. landings by domestic vessels have declined by nearly 60%. In 1985 only one small cannery was still operating in the United States, and the four major U.S. firms that supply 70% of the U.S. market are relying completely on tuna processed at offshore locations and canned tuna imports from Asia for their supplies.

When a U.S. industry with annual retail sales of $1.5 billion relocates to offshore territories and begins relying on foreign production, the economic impacts on the U.S. economy run deeper than the direct jobs and incomes lost in tuna-related industries. U.S.-based tuna operations are linked through their purchases and sales with many other segments of the U.S. economy. In general, each 100 tons of tuna landed and processed in the United States provides $15,000 in direct income to fishermen, an additional $12,000 in payments to cannery workers, and nearly $75,000 in income payments to workers in those U.S. industries that support domestic tuna harvesting and processing operations. The 200,000 tons of tuna landed in the United States during 1980 had an exvessel value of nearly $200 million, but by the time it was processed and packaged for market it was worth $400 million, had stimulated $1 billion in economic activity, and had generated 12,000 jobs and $300 million in household income in the United States. As U.S. tuna operations move away from the United States, these economic benefits are lost.

This project has resulted in publication of a California Sea Grant Working Paper, "The Economic Impact of Recent Changes in the Tuna Industry," that describes the results of this relocation.

Cooperating Organizations
American Tunaboat Association
Pacific Marine Fisheries Commission
Western Fishboat Owners Association

Publications
King, D. M. 1985. The economic impact of recent changes in the tuna industry. California Sea Grant College Program Working Paper No. P-T-47. University of California, San Diego, La Jolla.

Evaluating the Fatigue Behavior of High-Strength Concrete under Marine Conditions

University of California, Berkeley
R/NP-1-15A
Project Initiated: October 1, 1985
Project Completed: June 30, 1986

Ben C. Gerwick and Weston T. Hester

Marine structures have been made with reinforced and prestressed concrete for many years. They have been used for port facilities, coastal structures, and structures in the open sea, and have exhibited excellent performance when properly designed and constructed.

Sea structures are subjected to high-cycle (more than 10^5 cycles), low-magnitude (up to 65% of ultimate strength) wave action for their entire life span. In addition, these structures are subjected occasionally to high-magnitude loads such as breaking storm waves and the impact of floating ice and other objects. For a typical concrete sea structure, high-cycle fatigue has not been considered a significant problem. However, low-cycle, high-magnitude fatigue is now recognized as a source of degradation, and especially when there are numerous cycles into the tensile cracking range. Previous research done in Europe on fatigue behavior has shown that a few high-magnitude cycles initiated cracks, which were then reopened by the low-magnitude cycles, leading to accelerated fatigue failure, especially in the corrosive marine environment. It was also found that the submerged concrete was considerably more vulnerable to fatigue failure than the same concrete in air.

Current research underway in Norway and the U.K. has reported that even standard weight concrete, which was not cracked by structural loads, undergoes significant loss of fatigue endurance under water because of the effect of the water in the microcracks between the cement paste matrix and the aggregate. To date, light-weight aggregate concrete has shown no such degradation.

Practical solutions for enhancing fatigue resistance include the use of prestressing, provision of adequate percentages of steel area and confining reinforcement, and limitation on stress ranges and cracking under high-amplitude loadings.

Recent developments in concrete technology, such as the use of microsilica fumes to produce denser concrete with fewer microcracks, raise new questions as to fatigue behavior and also offer opportunities for improvement by eliminating most microcracks.

Because of the use of new and improved materials and construction methods for sea structures, we are faced with a situation where codes may be either too conservative or unsafe. The move towards higher strength and lower weight structural concrete for sea structures requires conclusive research into the fatigue behavior of these concretes.

Goals and Conclusions

The goals of the project were to assimilate the state-of-the-art knowledge from different fields dealing with fatigue of engineering materials and arrive at possible explanations and solutions to the above problems.

A comprehensive literature review led to the following conclusions:

(1) Fatigue failure is a result of formation and propagation of internal microcracks in the concrete, probably both in the matrix and on the aggregate/paste interface.

(2) The larger the frequency of vibrating load, the longer the life of low-cycle fatigue of concrete (Sparks, 1982; Hatano and Watanabe, 1971; Muguruma and Watanabe 1984).

(3) The effect of aggregate type on fatigue resistance was not shown with any certainty, although the theory of fatigue seems to favor light-weight aggregate concrete over standard-weight concrete of similar strength.

(4) Moisture content seems to have a considerable effect on fatigue strength, although the exact nature of this effect is not clear from the available data on tests done on concrete. Geologists recognized similar phenomena in natural rocks and minerals, and a large body of knowledge was developed to describe and analyze it. Waza, Kurita, and Mizutani (1980) found that the velocity of crack growth in water-saturated rocks was 2 to 3 orders of magnitude greater than on room-dried ones.

(5) Waagaard (1977) concluded that compressive strength did not seem to affect the number of cycles to failure as long as the relative compressive stress level was the same.

(6) Bannister (1978) tested the effect of the addition of 1% volume friction of wire fibers and found that the reinforcement fatigue resistance in seawater was improved by controlling the crack width in the concrete at the maximum stress in the cycle.

(7) Bannister (1978) concluded that fatigue corrosion plays an important role in fatigue deterioration in submerged concrete.

(8) The presence of water around concrete specimens tested under high-cyclic loading invariably reduces the fatigue strength of the concrete (Waagaard, 1982; Roper, and Hetherington 1982). It was speculated that this was the result of a wedging action of water in the cracks as a result of the loading.

(9) Reversible loading between tension-compression state will reduce fatigue life compared to compression-compression loading (Horii and Ueda, 1977).

(10) Tests by Paterson (1980)

confirmed that hydraulic pressure had no effect on fatigue life even though the presence of water reduced it.

Test Methodology

Two main methods are common in testing for fatigue under marine conditions: (1) high-cycle, low-magnitude loading of concrete specimens with or without simulated marine environment; and (2) low-cycle, high-magnitude loading of concrete specimens with or without simulated marine environment.

The first method is more representative of the actual conditions under which a marine structure has to perform. The loads of 50–60% of design loads are applied at a wave frequency of around 0.05–0.3 Hz (cycles per second). Since concrete may resist loads of that magnitude for 10E6 to 10E8 cycles, tests will have to run continuously for many years. In most situations, this is not acceptable, and various methods of acceleration have been developed. A very common one is the introduction of precracking to simulate a structure that underwent some kind of overloading and is subjected to normal sea loads (Rabbat et al., 1979; Paterson, 1980). A crack is a source of stress concentrations and serves to initiate corrosion and fatigue cracking. This method is rather satisfactory in its representation of real structures, even though the location and size of the crack are predetermined and may greatly affect the results.

Another widely used method of acceleration is the use of high-frequency loading (up to 20 Hz). It was tested by many researchers (Awad and Hilsdorf, 1974; Gray, McLaughlin, and Antrim, 1961; Raithby and Galloway, 1974; Sparks and Menzies, 1973) as a means to cut the testing time by one to two orders of magnitude. There is, however, a serious debate on the accuracy of that method and the conflicting results achieved under various testing conditions.

One of the main objections to accelerated tests is that they exclude the effects of corrosion, which may take months to produce damage under normal marine conditions. One may attempt to simulate the corrosion effect by using concentrated solutions of aggressive agents, but most researchers feel that it is not possible to properly correlate between these results and the normal long-term effect of seawater.

The second method of testing for low-cycle, high-magnitude fatigue, assumes that the low-magnitude fatigue is not a problem under these conditions and concentrates on the high-magnitude loads.

Kesler (1966) showed that rest periods of 5 minutes increased the fatigue strength by allowing the concrete to heal. Longer rest periods did not seem to add to that recovery.

Considering all the above, it was decided to test the effect of low-cycle, high-magnitude loading on the high-strength light-weight concrete and to test the possibility of using concentrated seawater. The following were taken into consideration:

(1) The purpose of this test is not to arrive at exact engineering formulas, but to compare the behavior under fatigue loading of various materials. As long as the testing method is consistent and the comparison is done under the same accelerated conditions, the results should be valid.

(2) The size of specimens has to be limited due to cost, capacity of the dynamic jack, etc.

(3) In choosing the appropriate cycling frequency two concerns were important. It should be as close as possible to the actual frequency of 0.05 to 0.3 Hz (Waagaard, 1977), but the testing time for each set should not exceed 2 weeks. A frequency of 1 Hz was chosen for initial testing, and a possible higher frequency of 6 Hz if 10E5 cycles will be exceeded.

(4) The actual loading sequence on marine structures in the ocean is random in nature. However, testing under these conditions would be technically more difficult, and would make it impossible to compare our results with dry specimens and existing fatigue data. Therefore, constant stress levels will be maintained.

(5) The maximum stress level will correspond to 85% of static ultimate strength.

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Economic Impact Models for Coastal Counties in California

University of California, San Diego
R/NP-1-15B
Project Initiated: December 1, 1985
Project Completed: March 1, 1986

Dennis M. King

The goal of the project was to develop county and regional economic impact models for twenty coastal California regions using an economic modelling system known as the IMPLAN system, which was developed by the U.S. Forest Service. The project was to result in a reference document that described the IMPLAN system and interpreted the results of the empirical analysis, which included Output, Income, Employment, and Value-Added Multipliers for each of up to 400 industrial sectors in each of the specified regions.

The project was completed in February, 1986 and the final project report was submitted to Sea Grant. For a number of technical reasons, the twenty regional models that were planned were eventually collapsed to eighteen regional models and one state model. Because of the amount of information produced from each model and the number of different models, the results for the statewide model were presented in the project report and were used to describe the approach. Results from the other regional models were submitted separately.

These IMPLAN models provide a low-cost and generally reliable method of developing impact multipliers for regional economies and have many useful applications. They have been used to generate economic information by the California Department of Fish and Game, the California Coastal Commission, the Pacific Fisheries Management Council, the Pacific Marine Fisheries Commission, and several central California coastal communities. Economic impact models developed by Sea Grant and the most recent application of the IMPLAN modelling system in this project have served as the empirical foundation for evaluations of commercial and recreational fisheries, offshore oil, fishing/oil conflicts, and fisheries management and are used and cited extensively by local, state, and federal agencies and private interests in their evaluation of marine-related decisions.

Cooperating Organizations

Pacific Coast Federation of Fishermen's Associations
Pacific Fisheries Management Council
West Coast Fisheries Development Foundation

Aging in Fishes: A New Technique Based on Age Pigments (Lipofuscin)

University of California, San Diego
R/NP-1-15C
Project Initiated: May 1, 1986
Project Completed: September 30, 1986

Russell D. Vetter

In fisheries management, age structured models are the preferred method for meeting the key objectives of estimating optimal yields and determining the effect of fishing on stock structure. However, few species of commercial marine fishes exist in which age can be determined with certainty. The determination of lipofuscin concentration in fish tissues may be an alternative method to age fishes that could validate existing techniques and could improve estimates of long-lived species where other techniques are difficult to apply.

With this research we have successfully accomplished two of the three goals that we proposed. (1) Lipofuscin was extracted and characterized for several organs in cold-water fishes. (2) A method for maximum extraction of lipofuscin in fish tissue was established. The third and final goal, that of establishing an unequivocal relationship between age and lipofuscin concentration, was only partially accomplished, as further research is needed in order to find a tissue or part of a tissue where lipofuscin accumulates at a faster rate.

Lipofuscin was successfully extracted and quantified from four different tissues of fishes. Wavelength of fluorescent excitation and fluorescent emission maxima of this compound in chloroform are presented in Table 1. All maxima are within the range cited by Sheldahl and Tappel (1973): fluorescence excitation maxima between 340 and 370 nm and fluorescence emission maxima between 420 and 470 nm.

Three methods were tested for maximum lipofuscin extraction. The first two methods (Tappel, 1975; MacArthur and Sohal, 1982) have chloroform:water (2:1) as extractive solvent. They differ in the optimal volume to weight ratio, temperature of extraction, and number of times the chloroform phase is washed with water. These methods were specifically developed for extraction of lipofuscin. The third method was developed for lipid extraction in fishes (Bligh and Dyer, 1959). Results of the test are displayed in Table 2. MacArthur and Sohal's method yielded higher fluorescent signal, so this method, with minor modifications, was adopted for subsequent quantitative studies.

Table 1. Spectral Fluorescent Characteristics of Lipofuscin in Chloroform for Two Species of Fishes, Rainbow Trout (*Salmo gairdneri*) and Dover Sole (*Microstomus pacificus*).

	Fluorescence Excitation Maximum (nm)	Fluorescence Emission Maximum (nm)
Rainbow trout		
heart	362	430
brain	380	440
liver	365	450
Dover sole		
heart	365	435
brain	365	430
liver	365	440
white muscle	350	460

Two sources of contamination were observed in the chloroform extract, as mentioned by Fletcher, Dillard, and Tappel (1973). Flavoproteins (wavelength of maximum fluorescence excitation at 288, 350, 450 nm and maximum fluorescence emission at 520 nm) were washed out of the chloroform phase with 2 rinses of water. Retinol (wavelengths of maximum fluorescence excitation at 325–340 nm and maximum fluorescence emission at 475 nm) was photo-oxidized by exposing the chloroform extract to UV radiation (250 nm). As expected, liver was the tissue with highest concentration of retinol, although it was also present in brain and heart tissues. For example, wavelengths of fluorescence excitation and emission maxima in liver of Dover sole (*Microstomus pacificus*) shifted from 352 to 365 nm and from 470 to 440 nm, respectively after UV radiation. Fluorescence in arbitrary units per mg of brain tissue.

Table 2. Comparison of 3 Methods of Lipofuscin Extraction*

	Fluorescent units	
	Average	Std. Dev.
MacArthur and Sohal (1982)	0.903	0.083
Tappel (1975)	0.596	0.078
Bligh and Dyer (1959)	0.416	0.096

*See text for details. Fluorescence excitation at 360 nm and fluorescence emission at 440 nm.

Quantitative extraction of lipofuscin from rainbow trout (*Salmo gairdneri*) in three different tissues (brain, heart, and liver) using Bligh and Dyer's method (1959) showed that concentration of extractable lipofuscin was constant for organisms ranging from 3 months to 3 years of age (n = 90). In the case of Dover sole, using the method of MacArthur and Sohal (1982), the concentration of lipofuscin per mg of brain tissue increased linearly with age for organisms from 5 to 25 years old (n = 8). On the average, 25-year-old fishes had twice as much lipofuscin as 5 year olds. We found that lipofuscin concentration

(in arbitrary fluorescent units) = 0.37909 ± 0.0221 (age of fishes, in years), r = 0.759, n = 13, degrees of freedom = 11. This increase is low compared to values seen in testis of mice where Tappel (1975) found a 14-fold increase in a 2-year interval.

Now that we have a suitable extraction method and have observed linear, albeit low, accumulation with age in brain tissue, we are presently analyzing other tissues that may accumulate lipofuscin at a more rapid, but still linear, rate. In summary, our results to date indicate that fish contain lipofuscin and accumulate it in a manner similar to mammals. Its use as a sensitive measure of age in fish will depend on our ability to find a tissue which shows a larger, but still linear, accumulation with age.

Cooperating Organizations

Aquatic Systems Inc., San Diego
California Department of Fish and Game
National Marine Fisheries Service, Southwest Fisheries Center
University of California, Davis

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Continuing Projects

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Continuing Projects: Sea Grant Extension Program

The Sea Grant Extension Program, which is administratively housed in UC Cooperative Extension, is the principal provider of advisory services for the California Sea Grant College Program. Since the program's inception in 1971, marine advisors and subject area specialists have actively encouraged the adoption and implementation of new technologies in port development, commercial and recreational fishing, aquaculture, seafood technology, coastal resource management, and marine education and recreation.

The program works closely with more than 200 cooperating organizations in government, industry, and academia to provide technical information and assistance.

Extension efforts are organized into four major program areas: Marine Fisheries, Coastal Resource Management, Seafood Technology, and Aquaculture.

Marine fisheries is the largest program area within the California Sea Grant Extension Program. All seven marine advisors have significant fisheries efforts in their regional programs. The advisors are supported by the marine fisheries specialist and a broad research base in universities and agencies. In addition. the Sea Grant Extension seafood technology program is closely linked to the marine fisheries efforts.

Recent state legislation made funds available for a low-interest loan fund and educational program directed at increasing fuel efficiency in the commercial fishing fleet. During 1985-1986, the fisheries specialist and a marine advisor worked with the industry advisory committee to select appropriate fuel-conserving technologies for testing. At-sea tests to measure increased fuel efficiency of "bison" trawl doors were completed, and at-sea tests to document fuel savings of self-polishing paints and optimum propellers were designed. Workshops were planned to provide information on fuel conservation and details of the state's loan program to fishermen statewide.

Commercial fishermen indicated a need to improve their record keeping and financial management. Thus, workshops were held on financial management, insurance, and lobster limited entry.

Dozens of fishing vessels and lives are lost at sea each year. At the same time, insurance costs have increased tremendously. The objectives of another Extension project were to have fishermen increase proper use of marine safety equipment and practices. As a result of workshops on safety practices, safety equipment was purchased by at least 2O percent of the participants, and 40 participants were trained in sea survival procedures.

A growing body of research indicates that albacore aggregate in fishable quantities along sea surface temperature and ocean color fronts. Thus, advisors posted sea-surface temperature charts regularly throughout California and used workshops and newsletters to inform fishermen about the products.

The northern California Marine Weather Relay Program was publicized, and workshops were held to inform mariners of the program. The program was terminated because of lack of participation, but then reintroduced in cooperation with the Coastal Fisheries Foundation.

Habitat restoration and resource enhancement have become important tools in restoring salmonid resources. California Sea Grant Extension personnel helped to organize the annual Oregon Salmon and Trout Enhancement Program Conference and the California Salmon and Steelhead Restoration Conference. A marine advisor completed two stream structure projects with a 4-H group on the Smith River. A fish weir was designed for evaluating the impact of a local hatchery project on Big Creek. Other advisors provided technical support to numerous salmon restoration groups in cooperation with state and local agencies. The Sea Grant Extension Program co-sponsored a training session on salmonid surveys with the American Fisheries Society.

During 1985-1986, a marine advisor completed the sixth of a ten-year study on chinook salmon spawning. His data will assist the California Department of Fish and Game to improve fisheries enhancement in the Smith River system.

Extension advisors investigated experimental trap fisheries for octopus, shrimp, and rock crab. A marine advisor completed a paper describing what is known of the development, utilization, and life history of the shark *Squatina californica* in south-central California.

Nearshore set net fishermen have lost significant fishing areas along the central California coast as a result of closures to protect marine mammals and sea birds. Advisory personnel conducted meetings with industry and agencies to identify alternative gear and methods. Marine advisors also cooperated with the Coastal Fisheries Foundation to develop a Saltonstall-Kennedy proposal for research on alternative gear.

The objective of another Sea Grant Extension project is to increase Vietnamese fishermen's awareness of safety practices and procedures. Vietnamese vessel owners were trained in the proper use of marine electronics equipment and safety equipment practices. Materials on these topics were developed in Vietnamese.

Sea Grant extension personnel work to increase the awareness of marine educators and the public about coastal and fisheries issues.

One marine advisor built an aquarium incubator to hatch steelhead eggs, and designed related classroom materials that were used in three elementary schools. Over 75 radio and TV spots were produced statewide on marine topics. A seafood shortcourse provided information to home economics teachers and others. Numerous training sessions for teachers, 4-H leaders, and other educators were held.

In cooperation with charterboat associations, a Saltonstall-Kennedy funding proposal was developed to determine angler perception of underutilized fish species and of fishing on commercial passenger fishing vessels.

Also, a research project on the adoption of technical innovations by commercial fishermen was completed and the results written up in the doctoral dissertation of the fisheries specialist. The results were used for staff training and for setting program priorities. They were presented to numerous industry, agency, and academic groups.

Coastal Resource Management is the second major program area within the California Sea Grant Marine Extension Program.

The overall objectives of a project on offshore oil development were to identify the impacts of expanded development on ocean resources and on marine related industries, and to improve inter-industry communication and conflict resolution efforts.

Two field experiments to study the effects of seismic acoustic signals on the eggs and larvae of anchovies were completed by contractors selected by the Eggs and Larvae Committee and the American Petroleum Institute. Three articles describing progress in this study were published in the *Oil and Gas Project Newsletter for Fishermen and Offshore Operators*. One marine advisor developed a slide presentation on the "offshore oil/fisheries communications and conflict resolution project" for use by National Sea Grant College Program staff and conducted a training session for Sea Grant Extension staff on related issues. Another marine advisor gave presentations on oil/fisheries conflicts to the Eureka Chamber of Commerce and the American Fisheries Society, and provided information to Minerals Management Service personnel. Still another improved the distribution of seismic survey notices in San Diego County to include trap and other fishermen who had not been receiving the information.

Sea Grant Extension personnel also assisted coastal counties and cities in developing projects to mitigate the impacts of offshore oil and gas development on coastal resources. For example, they cooperated in planning and hosting a Santa Barbara County Resource Management Department workshop to develop guidelines and potential projects for the use of County Fisheries Enhancement Funds; informed fishing industry repesentatives of the Coastal County and City Offshore Energy Block Grants through newsletter articles and personal contacts; provided Trinidad City and the Humboldt County Public Works Department with information for proposals for block grant funds; developed a comprehensive list of guidelines for use of offshore energy funds; and provided the information to the Humboldt County Board of Supervisors.

A marine advisor assisted the Northern California Chapter of the Women's Fisheries Network and the Army Corps of Engineers Bay Model Visitors Center in developing an educational forum on water quality in San Francisco Bay and its effects on fisheries resources. Another helped to organize a meeting for Cooperative Extension county staff and local agencies and organizations on statewide water policy issues, and served on the Cooperative Extension Water Task Force Committee to develop training programs.

In the area of port, harbor, and marine management, advisors completed a survey of marina managers and harbor masters to determine research and education needs; provided information on Sea Grant Extension Program projects to harbor masters and county property managers during Southern California Marina Lease Exchange Association meetings; participated in an interdisciplinary comprehensive port management study for the Crescent City Harbor District; and developed information on client relations, economic development opportunities, harbor operations, and property utilization.

A list of key contacts for San Diego, Los Angeles, and Orange counties was expanded. A Marine Advisory Committee was formed in San Diego County, and new members were appointed to the advisory committee for Los Angeles and Orange counties. A publication on "Ocean Related Employment and Careers" was published, and a seminar on the topic presented to public school teachers and specialists. Presentations and publications on marine projects were given to 4-H leaders and Headstart teachers. A new quarterly newsletter, *Tidelines*, was instituted in Southern California, and the publication *San Francisco Bay Recreational Opportunities and Climate* was revised and updated.

Elkhorn Slough is the second largest salt marsh in California, and was the first National Estuarine Sanctuary established in California. A marine advisor served on the Sanctuary Advisory Committee, which reviewed and made recommendations to the Department of Fish and Game on education and research programs for the slough and on area management, including the design of wetland restorations.

The third major program area, seafood technology, addresses the need to improve domestic seafood quality to compete with imported products and to provide consumers with higher quality products.

Cooperative Extension sponsored "Better Process Control Schools," sanitation workshops, a statistical quality-control short course, and a freezing-technology short course. A

Sea Grant Extension quality-control short course on caviar processing, sanitation, and quality control was held for quality-control technicians working in processing plants in China. A presentation on seafood quality and defects was given to institutional food buyers. Publications were prepared on the use of detergents and sanitizers in food plants.

Training on seafood safety, handling, nutrition, and home preservation was given to food professionals and Cooperative Extension staff. Spanish translations of four consumer seafood leaflets were prepared. The seafood technology specialist and a marine advisor designed a research project to evaluate handling techniques on commercial fishing vessels for fresh and frozen albacore tuna, and to develop guidelines for commercial albacore fishermen. Funding for the project was obtained from federal Saltonstall-Kennedy funds.

The objective of another project was to have seafood retailers improve their sanitation practices and their quality control and seafood handling techniques. During 1985-1986, six additional seafood retailing workshops were conducted by the University of Washington Sea Grant College Program, with funding from the Alaska Seafood Marketing Institute, and with assistance from California Sea Grant Extension specialists, advisors, and local seafood processors. There is a need to develop marketing alternatives for salmon and albacore. Sea Grant Extension projects—including workshops, news releases, promotional information, and publications—were been partially responsible for the increase in the market share for frozen albacore loins and specialty products between 1983 and 1986.

Seafood processing waste management continues to be a serious economic problem for processors. Meetings were held with seafood processors and agencies in northern California to determine if Sea Grant Extension can assist in the solution of existing processing wastewater handling and treatment problems.

The need for uniformity in naming seafoods and for minimizing mislabeling has been identified by the seafood industry, the California Department of Health Services, and consumers. Sea Grant Extension personnel are assisting the California Seafood Institute to develop guidelines for seafood retailers, restaurants, and food service establishments on preferred common names for seafood species, portions, and cooking methods.

Aquaculture, the fourth major program area, is a substantial activity in California, and is growing in importance.

Paralytic shellfish poison (PSP) monitoring stations have been established in the commercial shellfish growing areas of Drakes, Tomales, and Humboldt bays. News releases describing the monitoring program and the relation of the sports-harvest quarantine to commercially marketed shellfish are prepared each year and distributed to the news media throughout the state. News articles have been prepared to explain PSP, the annual quarantine, and the safety of PSP-monitored commercial shellfish.

A purified test diet for juvenile sturgeon was developed that will be used as a control diet for future nutritional research on sturgeon, and an additional grant was developed to study protein and other nutritional requirements of sturgeon. Information on nutritional requirements and diseases of sturgeon plus general culture technology was extended to agency hatcheries. A copy of the sturgeon hatchery manual will be be available in 1988.

Contacts were established between commercial fish growers and the UC Davis Disease Diagnostic Laboratory to provide diagnostic services to the industry. Relations were also established between County Extension staff and the diagnostic laboratory to facilitate rapid dissemination of diagnostic and disease information to clientele groups. Information on state disease regulations was developed by the Aquaculture Specialist while he was serving on the State Aquaculture Disease Committee, and this information was distributed statewide through the Aquaculture Extension newsletter.

The Aquaculture Specialist participated as a member of the California Department of Fish and Game Interagency Advisory Committee for Aquaculture Development to consolidate regulations governing aquaculture activities in the state. He also served as one of a three-person subcommittee to gather existing regulations of each agency impacting aquaculture, with the objective of consolidating these regulations into a single document.

Oyster aquaculture in California is valued at between $3 and 4 million and relies totally on the production of hatchery seed stock. Information on the progress of polyploid oyster research being conducted on the West Coast was extended to the shellfish industry by Sea Grant Extension, which also assisted commercial producers on remote setting of oyster spat and larvae as well as facility design.

Sea Grant Extension personnel made information on East Coast depuration technology available to the shellfish industry, along with information on Federal FDA interpretations of sanitation regulations. Interstate Shellfish Sanitation Program issues were distributed to industry personnel, and meetings established between agency and industry personnel to formulate a position for California at the National Interstate Shellfish Sanitation Program Conference. Funding was secured from the UC Davis Aquaculture and Fisheries Program and the Department of Agricultural Engineering to initiate depuration research.

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Cooperating Organizations

Ab Lab
Abalone, Inc.
Abalone Farms, Inc.
Alameda County Vietnamese Commercial Fishing Program
Alaska Sea Grant College Program
Alaska Seafood Marketing Institute
American Fisheries Society
American Golden Sea Co., Inc.
Anthony's Seafood Market
Aquaculture Association of Canada
Arcata Department of Public Works
Arcata Union
Arrowhead Fishery
Battelle Memorial Institute
Bennett's Fish Market
Berkeley Marina
Biomed Research Labs, Seattle
Bob Morrel Enterprises, Inc.
Bodega Bay Fisheries Marketing Association
British Columbia Mariculture
British Columbia Ministry of Environment
Brookings Fishermen's Marketing Association
Brookings Fishermen's Wives
California Abalone Association
California Academy of Sciences
California Aquaculture Association
California Assembly Office of Research
California Association of Harbor Masters and Port Captains
California Coastal Commission
California Coastal Operators Group
California Conservation Corps
California Cooperative Fisheries Research Unit
California Department of Boating and Waterways
California Department of Economic Development
California Department of Fish and Game
California Department of Food and Agriculture
California Department of Health Services
California Department of Motor Vehicles
California Department of Parks and Recreation
California Department of Transportation
California Energy Commission
California Energy Extension Service
California Energy Resources, Conservation and Development Commission
California Farm Bureau
California Fisheries Association
California Gillnetter's Association
California Marine Parks and Harbors Association
California Marine and Navigaiton Conference
California Maritime Academy
California Office of Planning and Research
California Sea Farms
California Seafood Institute
California Shellfish Company
California State Coastal Conservancy
California State Lands Commission
California State Parks
California State University, Chico
California State University, Hayward
California State University, San Francisco
California Urchin Divers Association
Calm Cove Oyster Company, Washington
Canadian Department of Fisheries and Oceans, Newfoundland Region
Candlestick Park State Recreation Area
Carmel River Steelhead Association
Castle Rock Fisheries
Catfish Farmers of America
Center for Education and Manpower Resources
Central Coast Hook and Line Association
Central Coast Seafoods
Chesapeake Fish Company
Coast Oyster Company
Coastal Fisheries Foundation
Coastal Service Corporation
College of the Redwoods
Commercial Fishermen of Santa Barbara, Inc.
Congressman Doug Bosco's Office
Connecticut Sea Grant College Program
Continental Shelf Associates
Cousteau Society
Crescent City Fishermen's Wives
Crescent City Harbor District
Crescent City Parks and Recreation
Cuesta College
The Cultured Abalone
Curry County Fishermen's Association
Dana Wharf Sportfishing
Del Norte County Board of Supervisors
Del Norte Fishermen's Marketing Association
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ERG Pacific
East Bay Fishermen's Association
East Bay Regional Park District
Eel River Restoration Project
Elkhorn Slough Foundation
Elkhorn Yacht Club
Eureka Federal Savings and Loan, Half Moon Bay Office
Eureka Fisheries, Inc.
Eureka Ice and Cold Storage
Eureka Times-Standard
European Aquaculture Society
F/V *Abrigo*
F/V *Ann*
F/V *Arnie P.*
F/V *Blue Pacific*
F/V *Gus D.*
F/V *Janus*
F/V *Kelly Ann*
F/V *Marion W.*
F/V *Sally Kay*
F/V *Salty Lady*
The Fish Tail Market
Fisheries Protection Institute
Fisheries and Oil Industries Joint Committee
Fisheries and Oil Industries Liaison

Office
The Fisherman
Fishermen's Cooperative Association
Fishermen's Marketing Association, Inc.
Fishermen's Wharf Seafoods, Santa Barbara
Fishermen's Union—I.C.W.U. Local 33
The Fishery
Fishing World Magazine
The Fishmarket
Five-Cities-Times-Press Recorder
Florida Sea Grant College Program
Friends of Del Norte
Friends of the Sea Otter
Gilroy Flycasters
Golden Gate Fishermen's Association
Golden Gate National Recreation Area
Governor of California's Office of Planning and Research
H & N Fish Company
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Heart of the City Farmer's Market
Hopkins Marine Station
Howorth & Associates, Santa Barbara
Humboldt Bay Fisheries Association
Humboldt Bay Harbor, Recreation, and Conservation District
Humboldt Bay Herring Company
Humboldt County Board of Education
Humboldt County Board of Supervisors
Humboldt Fish Action Council
Humboldt Fishermen's Marketing Association
Humboldt State University
Instutute of Food Technologists
International Paint Company
Island Packers
J.A.C. Creative Foods
J.J. Camillo Seafood Company
Johnson Oyster Company
Joint Committee on Fisheries and Aquaculture (Senator Barry Keene, Chair)
KIEM-TV
KVIQ-TV
Kamilche Sea Farms, Washington
Kerckhoff Marine Laboratory
King Harbor, City of Redondo Beach
Little Skookum Shellfish Growers, Washington
The Log
Long Marine Laboratory
Los Angeles County Board of Education
Los Angeles County Department of Beaches and Harbors
Louisiana State University Sea Grant Program
Louisiana Crayfish Farmer's Association
Marin Fish and Game Advisory Committee
Marine Associations Council of California
Marina City Club
Marine Mammal Commission
Maryland Oyster Growers
McDowell Valley Vineyards
Mediation Institute
Mendocino Fish Advisory Committee
Meyer Resources, Inc.
Michael Brandman Associates
Mid-Coast T.V.
Miller-Rellim Redwood Company
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Monterey Bay Salmon and Trout Project
Monterey County Health Department
Monterey Harbor, City of Monterey
Monterey Peninsula Flycasters
Morro Bay Commercial Fishermen's Association
Morro Bay Harbor Department
Moss Landing Commercial Fishermen's Association
Moss Landing Harbor District
Moss Landing Marine Laboratory
Moss Landing Women for Fisheries
Mt. Lassen Trout Company
NOYO Pride Fisheries
NOYO Women for Fisheries
National Environmental Satellite Data and Information Service
National Fisheries Institute
National Marine Fisheries Service
National Marine Manufacturers Association
National Ocean Service
National Oceanographic Data Center
National Weather Service
The Nature Conservancy
New Growth Forestry Services
N. J. Russo Co, San Pedro
New York Sea Grant College Program
North Carolina Sea Grant College Program
Northcoast Commercial Fishermen's Association
Northern California Indian Development Council
Northern California Marine Association
Northwest Ocean Sercice Center
Oakland Public Library
Ocean Fare Sales and Marketing
Oceanic Society
Oceanside City Harbor District
Office of Coastal Zone Management
Olympia Oyster Company, Washington
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Orange County Sheriff-Coroner Department
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Commission
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Santa Barbara Harbor Department
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South Carolina Sea Grant Program
Southland Farmers' Association
Southern California Coastal Water Research Project
Southern California Edison
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Southern California Marina Association
Southland Farmer's Market Association
Sportfishing Association of America
Sportfishing Association of California
Sportfishing Institute
The Squid Machine Corporation
State Fish Company
Sun Bulletin, Morro Bay
Supreme Seafoods
TV Station KOVR
Taiyo Fish Company
Taylor University, Washington
Texas A&M University Sea Grant College Program
Tideland Shellfish Company
Tom Lazio Fish Company
Trinidad Fishermen's Marketing Association
Umpqua Aquaculture, Inc., Oregon
U.S. Army Corps of Engineers
U.S. Coast Guard
U.S. Coast Guard Auxiliary
U.S. Department of Agriculture
U.S. Department of Interior
U.S. Department of Interior Minerals Management Service
U.S. Fish and Wildlife Service
U.S. Food and Drug Administration
U.S. Forest Service
U.S. Small Business Administration
U.S. Soil Conservation Service
U.S. Trout Farmers Association
United Anglers of California
United Anglers, Inc.
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University of California, Davis
University of California, Department of Naval Architecture
University of California, Santa Barbara
University of California, Scripps Institution of Oceanography
University of Delaware Sea Grant Program
University of Florida Sea Grant Program
University of Hawaii Sea Grant Program
University of Maryland Sea Grant Program
University of Rhode Island Sea Grant Program
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University of Washington Sea Grant Program
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Vietnamese Pacific Fishermen's Association
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Washington Aquaculture Council
Washington Association for the Valuation of Ecosystems
Washington Department of Ecology
Washington Department of Fisheries
Washington Department of Natural Resources
Washington Sea Grant College Program
Washington State University Cooperative Extension
Wayman Fish Company
Wecott Bay Gourmet Shellfish
West Coast Fisheries Development Foundation
Western Fishboat Owners Association
Western Oil and Gas Association
Western Sea Treat
Whiskey Creek Oyster Farm, Oregon
Wisconsin Department of Natural Resources
Women's Fisheries Network
World Mariculture Society

Communications

The Communications Office of the California Sea Grant College Program 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, 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 Program 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 20 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.

California Sea Grant supports strong, sophisticated research in marine science and technology. In 1985-86, for example, the Communications Office reported on 43 Sea Grant-sponsored research projects at eight of the nine campuses of the University of California and three campuses of the California State University system. The projects fell into the general areas of Coastal Resources, Aquaculture, Fisheries, New Marine Products, Ocean Engineering, and Marine Affairs.

The program director is the chief spokesperson for the program. His information dissemination and public relations activities are varied and range from Congressional testimony to presenting student awards.

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*. It is perhaps our major public information product, and we plan to publish it annually. 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—such as the Pacific Rim (1984-85).

The 1984-85 *Summary Report* was organized around the theme of California Sea Grant's role in the community of the Pacific Basin—a region that has become one of the most dynamic growth areas of the world. Because we believe that much of the vitality of California Sea Grant derives from our international associations, the publication focuses on those of our program's research and other activities that relate most strongly to the Pacific.

The 1985-86 *Summary*, titled *Sea Grant: A National Resource for Marine Research and Education*, examined the role of academic research and graduate education in determining our nation's economic competitiveness.

A third core publication is the *Biennial Technical Report*, 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. Those produced in the 1984-86 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 quarterly by the Publications Office) as well as to *Sea Grant Abstracts*, which is distributed nationally.

In 1985-86, the Information

Specialist distributed reprints of 74 journal articles and papers from published conference proceedings. In addition, she distributed six publications in the California Sea Grant series (produced by this department), and miscellaneous publications in a number of categories (see table), for a total of 105 different items, or 18,809 pieces. Addition of publication announcements, press releases, and awards announcements brought the number of pieces distributed to 33,493.

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 1985-86, there were 1,642 "unsolicited" requests for information or publications (i.e., not directly generated by our own publications announcements), bringing the total number of pieces distributed to 42,215.

DISTRIBUTION STATISTICS FY 1985-86

Category	*Number*
Reprints	74
Theses/Dissertations	6
Conference Papers	7
Sea Grant Series	6
Abstracts	4
MAP Publications	5
Miscellaneous	3
GRAND TOTAL	105

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, Rosemary. 1985. *California and the Pacific: Exploring the exclusive economic zone, A summary report of the California Sea Grant College Program, 1982-1984.* (R-CSGCP-016, 40 pages, 15 figures.)

Amidei, R. E. 1986. *California Sea Grant and the emerging Pacific age: A summary report of the California Sea Grant College Program, 1984-85* (R-CSGCP-018, 36 pages)

California Sea Grant College Program. 1985. *California Sea Grant 1985-86 Program Directory.* (R-CSGCP-017, 28 pages, 7 figures.)

California Sea Grant College Program. 1985. *Directory of academic marine programs, A guide to programs in the marine sciences at California colleges and universities.* (R-CSGCP-014, 148 pages, 14 figures, 2 tables.)

California Sea Grant College Program. 1986. *California Sea Grant 1986-87 Program Directory.* (R-CSGCP-019, 28 pages)

California Sea Grant College Program. 1986. *California Sea Grant biennial report, 1982-84.* (R-CSGCP-020, 266 pages, 66 figures.)

Sea Grant Technical Series

Abbott, I. A. and J. N. Norris, eds. 1985. *Taxonomy of economic seaweeds, with reference to some Pacific and Caribbean species.* (T-CSGCP-011, 184 pages, 43 plates.)

Anderson, K. E., ed. 1985. *Advances in aquaculture and fisheries research*: Report of a California Sea Grant symposium, May 18-20, 1983. (T-CSGCP-010, 56 pages.)

Amidei, R. E., ed. 1985. *Applications of remote sensing to fisheries and coastal resources: Report of a California Sea Grant workshop.* (T-CSGCP-015, 80 pages.)

Amidei, R., editor. 1986. *Rockfish: A focus for research? Proceedings of a California Sea Grant workshop.* (T-CSGCP-015, 80 pages.)

Dennis Hedgecock, editor. 1986. *Identifying fish subpopulations, Proceedings of a California Sea Grant workshop.* (T-CSGCP-013, 56 pages.)

John N. Heine, editor. 1986. *Blue water diving guidelines.* (T-CSGCP-014, 46 pages, 12 figures.)

Educational Series

Zedler, Joy. 1985. *Vegetación de la marisma: Ejemplos del Río Tijuana. (E-CSGCP-004, 40 pages, 31 figures.)*

Other

King, D. M. and H. A. Bateman. 1985. The impact of recent changes in the tuna industry. (Working Paper No. P-T-47, 30 pages.)

Education

Sea Grant's commitment to education and training activities in the marine sciences remains evident in the projects it supports for students at all levels, as well as for the general public.

The Trainee Program

Virtually all of the research projects supported by California Sea Grant involve at lease one graduate student trainee. During their training, students conduct independent marine research while working alongside University scientists and engineers in demanding and stimulating research environments. This new talent will be responsible for maintaining America's scientific and technological leadership in coming years.

In 1984-85 72 Sea Grant trainees worked with project leaders at 12 California universities and colleges. Another 67 trainees were supported in 1985-86. Most of these students worked on or completed graduate degrees during their traineeships.

A trainee who used molecular genetic techniques to improve the chitinase-producing ability of the bacteria *Serratia marcescens* points out that his traineeship allowed him to learn a great deal about the academic system of research. "This traineeship has given me experience in handling all aspects of a research project," he reports. "Not only was I responsible for creating research projects, but I was also responsible for setting some goals for the project. This last year of my traineeship, I was responsible for an undergraduate research student.... This was a good learning experience for me as a teacher."

Another trainee, who studied freezing-induced changes in fish tissue, says, "as a Sea Grant trainee and working on my Ph.D. research work, I am learning to design my experiment scheme through critical evaluation of published work and consideration of feasibility of different approaches in terms of available resources."

Isaacs Scholarship

The fourth John D. Isaacs Sea Grant Memorial Scholarship was awarded in 1985 to Michael Topolovac, a graduate of Torrey Pines High School in Del Mar, for his research on reducing drag on an unmanned submersible he had designed and constructed. The 1986 Isaacs Scholar, Steen G. Trump of McKinleyville High School, was recognized for his research on seasonal resettlement rates of intertidal organisms at Moonstone Beach. 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.

Marine Sciences for the Public

California Sea Grant sponsors a program of marine education (titled "California and the Pacific: Marine Sciences for the Public") through which it supports educational activities at five university-based marine facilities throughout the state.

The program at Scripps Aquarium, Scripps Institution of Oceanography (UCSD), has focused primarily on two goals identified as top priorities at a statewide needs assessment meeting held in 1985: (1) to promote the institutionalization of marine education and (2) to present current ocean issues in the larger context of their social, political, economic, and Pacific ramifications. The primary activity in 1986 was a Sea Grant-sponsored symposium for educators. Titled "A Fish in Your Wallet," the event included a symposium for educators and the interested public that was addressed by Dr. James Crutchfield from the University of Washington and a subsequent teacher workshop on the role of the ocean in the economy of San Diego. In addition to the workshop, Scripps staff were involved in a "UCSD Science Teacher Institute"—the first of what is to be an annual summer course to upgrade elementary teachers' science capabilities. And representatives from each of California's 15 Teacher Education and Computer (TEC) centers were invited to attend a 2-week "Science Curriculum Implementation Center Summer Institute," for which Scripps Aquarium instructors prepared workbooks in ocean science for classroom use. The Scripps education program also provided workshops for elementary teachers from San Diego city and county schools entitled "Exploring the Coastal Environment and its Resources." Several other outreach activities were also used to achieve the program's objectives.

The University of California, Santa Cruz program is making the transition from teaching students to teaching teachers. Sea Grant funds were used to hire University student coordinators, and emphasis was placed on developing curricular materials for teachers and on giving workshops. The program continued ongoing work with Long Marine Lab, Natural Bridges State Park, Santa Cruz City Museum, and the Santa Cruz County Schools. Special activities included designing and implementing teacher workshops; providing educational demonstrations at the annual Cabrillo College Teachers Faire and at the Long Marine Lab open house; completing a slide show on the intertidal zone; conducting training sessions for docents at Long Marine Lab; developing classroom activities in conjunction with the FOR SEA curriculum; and planning a junior high marine science curriculum for use in San Jose. A summer training program for teachers, the Marine Science and Ecology Institute, had to be cut in 1986 due to the uncertainty of funding.

The overall goal of the Moss Landing Marine Laboratories' education program is to increase public understanding of marine science and coastal marine environments. Each spring, the public is invited to visit the laboratories to learn about marine and coastal science research and the marine environment. Because the laboratory gets many requests for tours from schools and other community groups as well as from drop-in visitors, a program of week-end visitations has been implemented this year, which averages approximately 10 tours per month. The Laboratory also maintains an outreach program, responding to requests for speakers to visit local schools, community groups, and libraries. About 8,000 students annually receive slide presentations on marine research activities and higher education/career opportunities, and/or live animal demonstrations. Talks on special marine topics were also given to adult groups. Bilingual programs at the Monterey County Head Start facilities were included among the 1986 outreach projects.

The objective of the program at the University of California, Santa Barbara, was to promote the transfer of marine-oriented information into society. Its primary- and secondary-school outreach program (where students visited the UCSB marine facility) involved 5,400 students in 1985-86—and this represented only half of those who wished to participate. The program was also active in bringing marine education to the schools—through classroom programs, participation in Career Days activities, and assisting teachers in creating marine science units.

The Marine Sciences for the Public program at Humboldt State University sponsored a series of teacher workshops for grades 4 through 8, whose focus was on developing low-cost, hands-on activities for students. The workshops, held on two consecutive Saturdays and attended by 12 teachers, emphasized a holistic approach to marine science education, including marine geology, oceanography, marine life, and environmental science. During the course of the workshop, a number of activities were developed and supplemented with field trips and laboratory exercises. A comprehensive notebook of background material and activities arising from the workshop was sent out to schools in Humboldt and Del Norte Counties as well as to specific individuals who requested them. Also, during the 1986 fall semester, an extension class was taught at the Marine Lab for elementary-school children and their parents using workshop materials.

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1987-1988

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Resources Agency
State of California
Sacramento, California

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