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CALIFORNIA SEA GRANT

Biennial Report of Completed Projects 1992-94

A Publication of the California Sea Grant College System

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

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Biennial Report of Completed Projects

1992–94

California Sea Grant College System
University of California
La Jolla, CA 92093
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TABLE OF CONTENTS

New Marine Products

Ocean Engineering and Instrumentation

Marine Affairs

Rapid Response

Introduction

This biennial report presents the results of research activities undertaken by the California Sea Grant College System during fiscal years 1992–94.

It is meant to be a technical record of our accomplishments for use by individuals in academia, government, and industry.

This publication contains only reports of completed projects (as opposed to descriptions of work in progress). It thus forms an important historical record of program achievement and an important document in terms of program accountability.

For readers unfamiliar with our program, the California Sea Grant College System 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."

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

James J. Sullivan
Director

Coastal Resources

Paralabrax as a Model for Predicting Effects of Ocean Warming

University of California, San Diego
R/CZ-94
1990–93

Richard Rosenblatt and Jeffrey Graham

The goal of this 3-year study was to determine the possible impact of global warming on the coastal fishes of Southern and Baja California. This narrative describes the initial results of the study.

The sea bass genus *Paralabrax* (Serranidae) was chosen as a model for the study of effects of temperature change on coastal fishes because the three species found locally form populations inhabiting a broad range of temperatures. *Paralabrax clathratus* and *Paralabrax nebulifer* are found from Point Conception to Magdalena Bay, Baja California. *Paralabrax maculatofasciatus* is found from Point Conception southward along the Baja California coast and also has a disjunct population in the Gulf of California.

The goby genus *Gillichthys* (Gobiidae) was chosen as a further group for study because it too has a species with a disjunct distribution. *Gillichthys mirabilis* occurs both within the Gulf of California and along the outer coast north to San Francisco, whereas its congener *Gillichthys seta* occurs only in the rocky intertidal regions of the northern Gulf of California.

In this study, we attempted to determine how local populations of these species will adapt to climatic changes and whether more southerly populations will gain a competitive advantage as temperature changes affect distributions. We examined biochemical, genetic, and physiological parameters that are sensitive to temperature in order to determine the ability of each population to withstand expected temperature changes and the consequent changes in interspecific competition.

Biochemical Analyses

Biochemical analyses included examination of the kinetics of lactate dehydrogenase (LDH), an important enzyme in the energy-producing glycolytic pathway. The activity of this enzyme is measured in terms of K_m, which assesses how well the enzyme binds its substrate. For each of the *Paralabrax* species, including the disjunct Gulf population of *P. maculatofasciatus*, K_m of LDH was measured in the range of 20–30°C. Results indicate that *P. nebulifer* has the most temperature-sensitive LDH, as its K_m increased almost twofold across that temperature range. The K_m of *P. maculatofasciatus* LDH is least affected by temperature across this same range; it increased approximately 50%. Some results suggest that the LDH of Gulf *P. maculatofasciatus* is more warm adapted than is the LDH of coastal Pacific *P. maculatofasciatus*. The LDH of the Gulf population had lower K_m values at each temperature throughout the range. The LDHs of the *Gillichthys* populations were examined during the final year of this project. The range of temperatures examined in this segment of the study was 10–40°C, reflecting the broad range of temperatures these populations encounter in their natural habitats. Results indicated that both *G. mirabilis* populations have similar K_m values across this range of temperatures, although the Gulf population inhabits a warmer and more variable thermal environment. *Gillichthys seta*, which occurs in the very warm rocky intertidal regions of the northern Gulf of California, has a much more thermostable K_m than do the *G. mirabilis* populations, indicating that the former is biochemically adapted to a much broader range of temperatures.

Genetic Analyses

Genetic relationships among the populations and species of *Paralabrax* were determined by using allozyme electrophoresis. With this method, we examined differences in proteins (allozymes) among groups and used the results to determine underlying differences in genes. The population genetic relationships among the Gulf and coastal populations of *P. maculatofasciatus* were examined by using 44 presumptive gene loci. Additionally, through collaboration with the National Marine Fisheries Service and Case Western Reserve University, we were able to examine mitochondrial DNA data further to address the question of genetic divergence between the Gulf of California and outer coast populations of *P. maculatofasciatus*, as well as among the three *Paralabrax* species as a whole.

In the allozyme part of the study, 40 of the 44 presumptive gene loci were used (the other four were general proteins). Mean heterozygosity and the percentage of polymorphism agreed with previous estimates based on allozyme data for marine fishes, including *Paralabrax* (Waples and Rosenblatt, 1987; Graves et al., 1990). The Gulf population of *P. maculatofasciatus* had six unique alleles, and three alleles in the San Diego sample were not found in the Gulf sample. The mean Fst value (averaged across all polymorphic loci) for differences in heterozygosities among the two subpopulations in comparison with overall heterozygosity as a measure of genetic drift was 0.021, indicating little genetic variation between the two sites. The Nei's (1972) genetic distance separating these populations is only 0.004, and the modified Roger's distance (Wright, 1978) is 0.061. These values suggest relatively high gene flow across the southern tropical region of the Gulf. In contrast, the Nei's genetic distance separating *P. maculatofasciatus* from *P. nebulifer* is 0.186, and the modified Roger's distance is 0.397.

The DNA analysis has been partially completed. We are analyzing approximately 500 DNA base pairs from the mitochondrial control region (including the highly variable D-loop) of three species of *Paralabrax*. We isolated and purified the DNA from the samples, including all samples analyzed in the allozyme part of this study. The DNA was then amplified by using a polymerase chain reaction, sequenced by using the Sanger dideoxy method, and analyzed by using polyacrylamide gels. Preliminary data for *P. clathratus* and *P. maculatofasciatus* are given here. One of the results was the finding that the sequence divergence among species of *Paralabrax* in the control region is considerably greater than that predicted on the basis of other congeneric comparisons, such as among species of the pleuronectid genus *Microstomus* and the scorpaenid genus *Sebastolobus*. High sequence divergence between these two species is making alignment difficult in the more variable regions. We have found three base transitional substitutions in 337 base pairs of five individuals of *P. maculatofasciatus*. None of the base substitutions were unique to the specimen analyzed from the Gulf of California population. However, its haplotype at the variable bases has not been found in any of the individuals sequenced to date from San Diego. Two individuals from San Diego appear to have the same haplotype. Thus, among the four individuals sampled from San Diego, we found three unique haplotypes.

Physiological Analyses

Effects of temperature change on physiological processes in *Paralabrax* were examined in three separate studies. Metabolic rate, hemoglobin-oxygen (Hb-O_2) affinity, and final temperature preference have been examined in relation to temperature change.

First, a computer-controlled respirometer was developed to measure oxygen consumption rates of individual fish as temperature is stepped from 12°C to 32°C. By examining the rate of increase in oxygen consumption with respect to temperature, an understanding of the underlying ability of each *Paralabrax* species to withstand temperature change can be derived. The findings indicated that *P. clathratus* and *P. maculatofasciatus* from San Diego both increase their oxygen consumption more slowly with temperature than does *P. maculatofasciatus* from the Gulf of California. This indicates that the two coastal species may be more eurythermal (able to tolerate a wider range of temperatures) than is the Gulf population.

Second, a method termed biotonometry was used to collect data on the Hb-O_2 affinity of each species and population of *Paralabrax* being studied. Results indicated that the different groups of *Paralabrax* have little difference in Hb-O_2 affinity across the temperature range of 12–33°C. Similarly, measurements of the blood oxygen capacity of each group showed no significant difference between any of the populations or species.

Finally, a thermal shuttlebox was developed to determine final temperature preferences among the groups of *Paralabrax*. Final temperature preference gives a general indication of the thermal optimum for a population, because the temperature the organism chooses when provided a large thermal gradient is assumed to be the temperature at which the majority of physiological functions are optimized (Reynolds and Casterlin, 1979). Thus, by comparing thermal preferences across populations and species, environmental temperatures necessary for the growth and survival of each species can be deduced.

Results from this study again suggested that little difference exists between the Gulf and coastal populations of *P. maculatofasciatus* or between these groups and *P. nebulifer* and *P. clathratus*.

Results and Accomplishments

The results of all the studies described here indicate that although the marine shore fishes of coastal California and the Gulf of California experience distinct thermal environments, they do not necessarily adapt differently to those environments physiologically, biochemically, or genetically. By using closely related but disjunct conspecific and congeneric pairs, we have been able to separate effects of ecological and phylogenetic divergence from adaptational differences induced by various thermal environments. In this way, we have shown that the disjunct populations of *P. maculatofasciatus* have no significant physiological differences in thermal adaptation and that their biochemical responses to temperature change are similar. The existence of unique alleles in the Gulf of California population of *P. maculatofasciatus* suggests either the elimination of these alleles by selection from the outer coast population or, less likely, one-way gene flow. The disjunct populations of *G. mirabilis* also show no difference in biochemical adaptation to a wide range of temperatures.

These findings suggest that in the face of warming due to anthropogenic release of greenhouse gases, displacement of northern forms by southern ones will not be a major threat, especially for relatively eurythermal species such as *Paralabrax* and *Gillichthys*. Further, these groups in the past have been able to thrive across a wide range of thermal environments without resorting to biochemical or physiological adaptations. Thus, if temperatures rise, these groups may be able to maintain populations in their present locations through phenotypic plasticity or appropriate behavior.

Cooperating Organizations

Centro de Investigagiones Cientificas y de Estudios Superiores de Ensenada, Baja California, Mexico

National Marine Fisheries Service, La Jolla

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Lectures and Conferences

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Fields, P.A. Effects of climatic change on the coastal marine biogeographic provinces of the western United States. Presentation given at INCOR conference, May 1993.
Rosenblatt, R.H. Ichthyological research in the Gulf of California. Plenary Address, Fifth Latin American Congress of Marine Biology, September 1993.

Detection and Monitoring of Bacteria with Molecular Probes

University of California, Los Angeles
R/CZ-104
1991–93

David J. Chapman and Clifford F. Brunk

Conventional analysis of aquatic systems for microbial pollution involves either counting individual microorganisms or selective culturing to detect specific groups of pathogens (e.g., fecal coliforms) or taxonomic groups (e.g., *Vibrio* spp.). These methods are subject to qualitative and quantitative errors. Counting individual microbes is time-consuming and provides little information about the bacterial species present. Culturing bacteria from natural samples is also time-consuming, and many species are missed because of their inability to grow under laboratory conditions.

The bacteria present in a population can be characterized by analyzing their small subunit (SSU) ribosomal RNA (rRNA) genes. Each bacterial species has a unique SSU rRNA sequence. This sequence can be determined by using DNA sequencing or analysis of restriction-fragment length polymorphism (RFLP). This type of approach has been used to analyze the bacterial population of the North Central Pacific Ocean (Schmidt et al., 1991), enumerate *Agrobacterium* in soil (Picard et al., 1992), and test food for contamination by *Listeria* (Wang et al., 1992).

We have made substantial progress in adapting these molecular techniques so that we can use them to detect point-source pollutants in coastal oceanic waters. We are looking for basic pollutant generic types against a background population of nonpolluting marine bacteria, without attempting to identify every taxon present in the marine profile. Our work for the past 2 years has involved the natural bacterial population of Santa Monica Bay, "controlled" test mixtures of marine bacteria (*Alcaligines aquamarinus*, *Pseudomonas nautilus*, *Oceanospirillum vaga*, *Flavobacterium okeanokoites*, *Vibrio alginoliticus*, and *Deleya marina*), and the typical polluting fecal coliform *Escherichia coli*. We have paid particular attention to developing protocols and procedures that are accurate and have a high degree of repeatability. We are also aware that speed and reasonable cost are considerations if the methods are to be used as standard assays.

In essence, our protocol involves preparing total DNA from a marine sample. The SSU rRNA genes in this DNA are amplified by using a polymerase chain reaction (PCR) (Mullis and Faloona, 1987). This eliminates the necessity of growing the bacteria under laboratory conditions. A primer with a fluorescent label or marker is used in the PCR amplification. The labeled SSU rRNA genes are treated with an appropriate enzyme (a restriction endonuclease), and an RFLP analysis is done. For the RFLP analysis, capillary electrophoresis and a laser-induced fluorescence detector are used.

The PCR requires only a small sample of seawater. In natural unpolluted waters, we have found that the bacterial population present in 15 l is sufficient to provide a signature profile. Sampling is done from a drifting boat and involves collecting 150 l of seawater over a 15-min interval. The sample is collected from surface (10 ft. or 3 m depth) waters by peristaltic pumping. From this, a 15-l aliquot is filtered through a 10-μm screen to remove plankton and sediment and then through a 0.22-μm nylon filter 90-cm in diameter to trap bacteria. Except for heavily polluted waters (e.g., drain runoff from Ballona Creek), this can be achieved by using a single tandem filtration. With polluted waters, the 15-l aliquot must be divided into smaller samples. The bacteria are removed from the wet filter, and their total DNA is extracted. Because the fluorescently labeled primer is so sensitive, samples as small as 3 pg of DNA can be detected after PCR amplification.

Recent studies (Tsai et al., 1991) have shown that not all bacteria are susceptible to lysis by lysozyme and detergents. Therefore, we have incorporated a freeze-thaw step into our protocol. Several times during the lysis phase of the DNA preparation, the bacterial cells are cycled between immersion in a mixture of dry ice and ethanol and immersion in water heated to 65°C. Virtually all the bacterial cells we have tested became fragile after exposure to extreme temperatures and were much more susceptible to lysis with lysozyme and detergents. The cells in the sample must be lyzed as completely as possible in order to get an accurate representation of DNA in the biomass. The buffer we use effectively prevents degradation of the DNA by nucleases.

Originally, we proposed using a radioactively labeled probe in the RFLP analysis. However, capillary electrophoresis coupled with fluorescence detection offers a number of distinct advantages over the radioactive approach. We therefore modified our protocol as follows:

Two primers were synthesized: an unlabeled primer and a primer with a fluorescent label. These primers should attach to the extreme ends of the SSU rRNA genes from virtually all eubacteria (Dams et al., 1988). The labeled primer attaches to one end, and the unlabeled primer attaches to the other end. Our preliminary work with a number of genera of marine bacteria and coliform bacteria indicates that the primers work well for amplification of a wide variety of eubacterial SSU rRNA genes.

The labeled PCR-amplified DNA molecules are treated with enzymes and analyzed by using a capillary electrophoresis unit equipped with a laser-induced fluorescence detector. Only minute amounts of sample (in the picogram range) are required for this step, and high-resolution separation is routine. A typical analysis at this step requires only about 30 min. The equipment can be easily automated for precise and reproducible quantitative analysis. The output is in the form of a computer file, which is ideal for comparative analysis and can be easily shared with other agencies. Using fluorescently labeled primers not only reduces the time required for RFLP analysis, but also allows us to avoid handling radioactive materials and the waste problems involved. This protocol is simple, accurate, fast, and cost-effective.

Treatment of SSU rRNA with restriction enzymes provides a specific signature (Avaniss-Aghajani et al., 1994). Work with mixture of four test bacteria and a combination of enzymes showed that a distinctive profile can be achieved by using only two restriction enzymes. Because of the size of an SSU rRNA gene (about 1600 base pairs), enzymes were chosen to maximize the number of fragments produced.

We are currently refining the PCR step to determine which parameters affect the amplification when different types and amounts of bacterial DNA are used in combination with various different primers. Optimization of the amplification step is crucial because it will allow us to choose the best primer combinations for samples collected from different sources. A good deal of effort is also being devoted to developing internal controls that will ensure the accuracy and reproducibility of our analysis protocol.

Throughout the past year, we have also been collecting and analyzing a data base of all bacterial SSU rRNA sequences that have been published to date. We have collected close to 1400 sequences and are continuing to add to our list. Computer simulations with this data base allow us to "predict" the patterns that would result from analysis of any given collection of bacterial DNA amplified with any specific set of primers and analyzed with any set of restriction enzymes. This approach should allow us to match results from unknown samples to results obtained from the computer data base and thus identify the bacteria present in the sample.

The combined use of PCR amplification of SSU rRNA genes and fluorescent capillary electrophoretic analysis of SSU rRNA sequences provides us with a powerful protocol that circumvents most of the problems associated with conventional methods for examining bacterial faunas. We anticipate that application of our protocol will provide a model for determining profiles of the bacterial faunas of coastal waters. With this protocol, we should be able to estimate the extent and dissemination of bacterial pollution from various sources and identify the specific bacterial taxa responsible.

Cooperating Organizations

City of Los Angeles Hyperion Sewage Treatment Facility
Orange County Sanitation District

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Methods to Improve Restoration of Pacific Estuarine Ecosystems

San Diego State University
R/CZ-106
1991–94

Joy B. Zedler

Southern California's natural cordgrass (*Spartina foliosa)* marshes support nesting of the endangered light-footed clapper rail (*Rallis longirostris levipes)* and a diversity of invertebrates. Constructed cordgrass marshes have not attracted rails to nest and are not functionally equivalent to natural marshes. Connector Marsh is the older constructed marsh at Sweetwater Marsh National Wildlife Refuge in San Diego Bay; it was transplanted to cordgrass in 1985. Marisma de Nación was transplanted in 1991. Clapper rails have not yet nested in either marsh. The lack of nesting has been attributed to shorter cordgrass canopies than are found in natural cordgrass marshes used by clapper rails (Zedler, 1993). The higher tides completely inundate the short vegetation, leaving no cover above water for use by birds or other nonaquatic marsh animals.

Connector Marsh had significantly lower levels of nitrogen in the soil than the adjacent natural marsh at Paradise Creek, suggesting a causal link between low levels of nitrogen, a shorter canopy, and the lack of nesting by clapper rails. Nitrogen is a limiting nutrient in salt marshes (Valiela et al., 1976; Covin and Zedler, 1988), but the role of organic matter as food for marsh consumers and as a supply of nitrogen for plant growth is not clear. Both nitrogen fixation and denitrification rely on organic matter as an energy source. Additions of organic matter stimulated nitrogen fixation in both natural and constructed marsh soils along San Diego Bay (Zalejko, 1989).

We used two approaches to improve methods of restoring and constructing coastal wetlands. To determine how rapidly nutrient pools have developed in the coarse substrates, we compared soil nitrogen pools in Connector Marsh as it aged from 6 to 10 years (1989–1994) with pools in an adjacent natural marsh remnant, Paradise Creek. Next, we developed experiments to test various amendments that might increase soil nitrogen pools.

Our long-term sampling of sediments in the Connector Marsh showed that organic matter pools are developing very slowly in this constructed marsh. Levels of organic matter in sediments increased about 0.5% per year, from approximately 4–5% during early studies (when the marsh was 3–4 years old) to about 8% in 1994 (at age 10 years; data for north islands). It is not clear whether the high value in 1994 is an anomaly or part of a continuing upward trend. Relative to Paradise Creek, the amount of organic matter in sediments in the constructed marsh appears to have increased, but the mean is still less than 75% of that in the natural marsh.

Despite improvement in the amount of organic matter in sediments, the soil in the constructed marsh has not accumulated nitrogen, and total nitrogen levels have remained close to 1 mg/g since 1988. Concentrations of total nitrogen in sediments in the Connector Marsh have been about 50% of the levels in Paradise Creek over the entire study period.

Through field experimentation, we tested the hypothesis that development of a constructed ecosystem would be accelerated by adding organic matter and nitrogen to the substrate. A newly excavated site, Marisma de Nación, was made available for experimentation before broader transplanting in 1991. We rototilled organic matter and nitrogen into the sediment (Gibson et al., 1994) and expected cordgrass to show increased growth in proportion to the amount of nitrogen added, whether from inorganic or organic sources. We further expected that nitrogen pools would increase after additions of organic matter (especially additions with low carbon-to-nitrogen (C:N) ratios) and that inorganic nitrogen fertilizer would also increase nitrogen pools, but for a shorter period. The field experiment was set up in Marisma de Nación immediately after its construction (February 1990). Additions of organic matter and nitrogen included straw (C:N = 84), alfalfa (C:N = 12), and ammonium sulfate (inorganic nitrogen). Cordgrass was transplanted to the experimental plots.

Rototilling alone appeared to have an adverse effect on growth of cordgrass, but rototilling organic matter into the substrate rapidly stimulated cordgrass growth. Effects on plant biomass were evident as early as 2 months after planting. Highest growth was obtained when alfalfa was added, either with or without inorganic nitrogen. Adding inorganic nitrogen to straw increased growth significantly more than straw alone (Gibson et al., 1994; Figure 1). Overall, the growth data indicate that alfalfa caused the most growth because it contained more nitrogen than the other treatments.

Relatively little of the nitrogen added was recovered by the aboveground plant material (Gibson et al., 1994). A substantial fraction of the readily mineralizable nitrogen was leached or denitrified during the month preceding planting. Results of a litter-bag decomposition assay in 1992 indicate that as much as 80% of the added nitrogen can be mineralized in the first month (Gibson, 1992). Because destructive sampling was not compatible with the long-term objectives of this study, we did not estimate

nitrogen storage in belowground biomass. Even if roots and rhizomes had eight times the aboveground biomass (Valiela et al.,1982), the fraction of nitrogen retained by the vegetation was small (Figure 2).

In the first year of the experiment, nitrogen-rich organic matter (alfalfa) increased plant growth but not the amount of organic matter in sediments and in nitrogen pools (Gibson et al., 1994). It was no surprise that in the second year, plant growth was still limited, even though treatment effects were still visible (Figure 1). The highest cordgrass biomass developed in treatments with alfalfa additions. But the maximum in 1991 (about 0.5 kg/m^2) was only about half the standing crop of a natural cordgrass marsh (approximately 1 kg/m^2, data of Winfield, 1980). Less than 5% of the nitrogen added was recovered in aboveground biomass, suggesting that leaching and denitrification rates were rapid on constructed salt marsh soil.

Expanding on this study, we asked how long nitrogen is retained in soils and how soil texture affects the results. We investigated the effects of a variety of amendments (manure, kelp, alfalfa, and inorganic slow- and fast-release fertilizers) on clay and sandy salt marsh soils. In the same experiment, we also tested waste products (kelp wrack, horse manure) as novel ways to enhance the nutrient pool more effectively and cheaply than by adding alfalfa.

This second field experiment was established in March 1992 in a disturbed area adjacent to Paradise Creek Marsh, at an elevation judged suitable for cordgrass. A completely randomized block design with 10 treatments and four blocks was used. Treatments included a low dose of alfalfa, a high (double) dose of alfalfa, kelp, horse manure, fast-release nitrogen (ammonium sulfate), slow-release nitrogen (sulfur-coated urea), alfalfa and clay, and fast-release nitrogen and clay. There were two controls: clay control and sand control. The mass of nitrogen was held constant at 96 g/m^2 for all treatments except

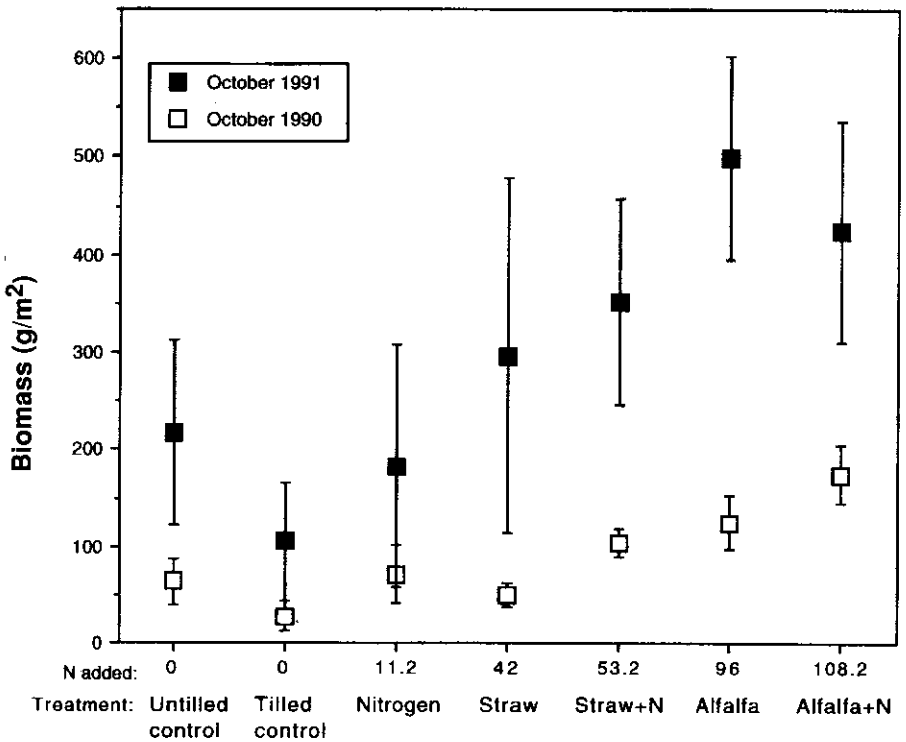

Figure 1. Cordgrass biomass (estimated from data on total stem length) during the first and second years after amending soils with organic and inorganic nitrogen (N). Modified from Gibson et al. (1994). Plants grew better with the addition of large amounts of nitrogen (*x* axis shows grams of nitrogen added) and did better in year 2 after vegetative expansion, yet the highest standing crops had only half the biomass of natural cordgrass marshes.

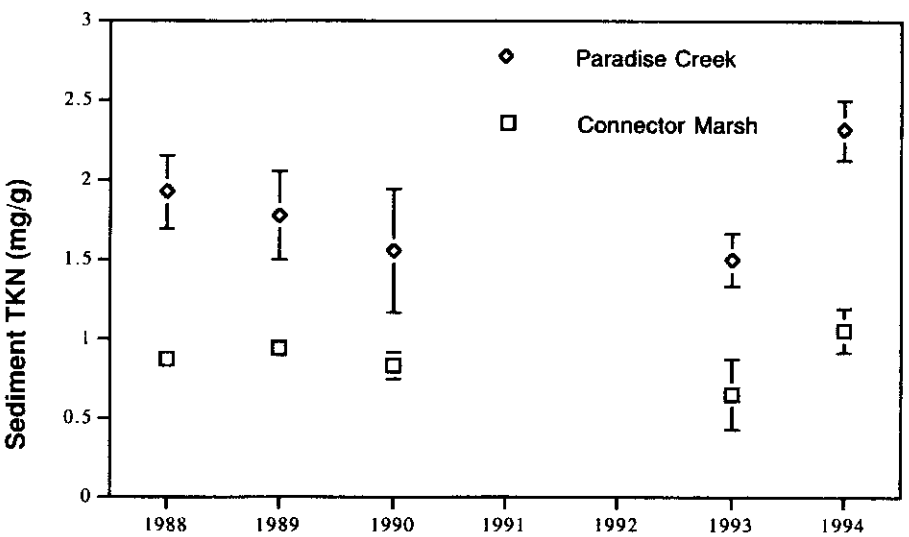

Figure 2. Mean total Kjeldahl nitrogen (TKN) in sediments over time in Paradise Creek (natural marsh) and Connector Marsh (constructed marsh). Number of samples was 3–8; error bars represent ± 1 standard error.

the controls and the double quantity of alfalfa (nitrogen = 192 g/m^2). Each plot was 0.5 m^2, separated from its neighbor by buffers of about 1 m.

We hypothesized that nitrogen would be retained more readily by finer than by sandier soils. Existing sediments were sandy: 83.5% sand, 10% clay, and 7.5% silt. Sediment with finer texture (50% sand, 30% silt, and 20% clay) was transported from a nearby site and placed in plots excavated to 20 cm on the experimental site. Sediment cores were analyzed for amounts of total nitrogen, KCl-extractable ammonium, and nitrate-nitrites, and percentage of organic carbon. Permanent wells (20 cm deep by 2 cm in diameter) were used to collect porewater for measurements of pH, salinity, extractable ammonium, and nitrate-nitrites. Data were analyzed by using two-factor ANOVAs, with block and treatment as the factors. Fisher's protected LSD test was used for planned pairwise comparisons (Day and Quinn, 1989). Data analysis was complicated by various problems, some beyond our control. A major hydrological disturbance occurred early in the experiment, when the tidal channel was intermittently closed by a construction crew. Variability was high within treatments, perhaps because buffers between treatment plots were narrow; leached nitrogen may have moved between adjacent plots.

Nitrogen was released in a pulse and was rapidly lost from both fine and coarse soils and for both organic and inorganic amendments, as indicated by amounts of total nitrogen at 0.5 and 1 week after fertilization (Figure 3). Variability was high and appeared to obscure any effects of treatments; no significant differences were found. At 0.5 week, the fast nitrogen-plus-clay treatment had the highest mean amount of total nitrogen (3.6 mg/g soil), which compared favorably with maximum levels found in local natural marshes: 3.01 mg/g in Paradise Creek (Pacific Estuarine Research Laboratory, unpublished data) and 2.38 mg/g in Tijuana Estuary (Langis et al., 1991). However, after that first sample, levels of total nitrogen

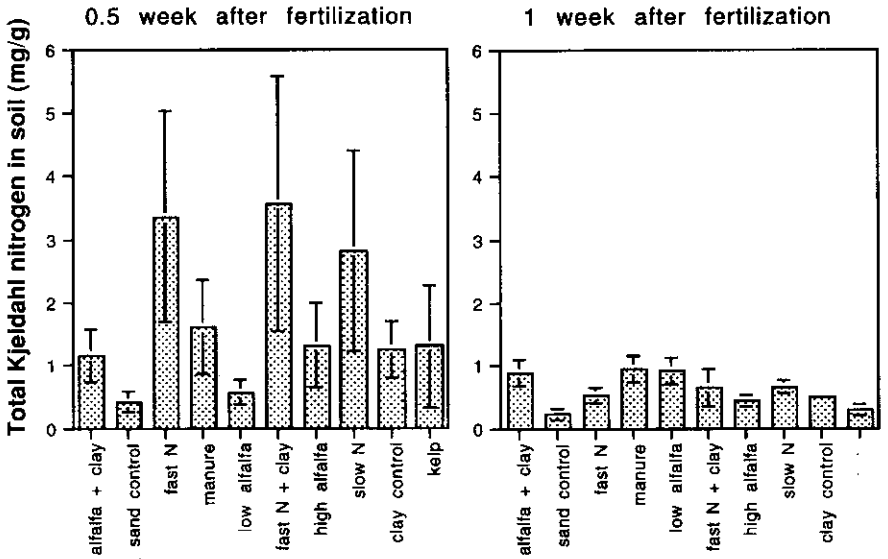

Figure 3. Mean total Kjeldahl nitrogen in soil after additions of inorganic and organic nitrogen (N). Four samples per treatment group; error bars represent ± 1 standard error.

Table 1. Grand Means of Total Nitrogen in the Substrate After Amendment with Organic and Inorganic Fertilizers

Time Since Amendment	Grand Mean	Standard Error
0.5 week	1.73	0.35
1 week	0.61	0.08
1 month	0.53	0.05
4 months	0.35	0.04
5 months	0.37	0.05
1 year	0.49	0.05

Note: Means include the eight nitrogen-addition treatments (see text) and two controls (*in situ* sediment and finer-textured sediment).

dropped to less than half the initial levels (Table 1). Amounts of total nitrogen in the soil showed no patterns after the first week, as the rank of means changed from date to date. The texture of the soil did not appear to have an effect on nitrogen retention, suggesting that higher concentrations of clay are needed. In all cases, the amount of total nitrogen present 1 year after treatment was considered inadequate from a biological perspective.

Because one-time amendments had no discernible long-term effect, additional studies were developed to assess the effects of multiple applications of nitrogen (on complementary projects). Wider buffers (2 m) are being used to minimize exchange of nutrients between treatment plots. Nitrogen is being added biweekly over the growing season. We hypothesize an improvement in the growth of cordgrass and slow but steady development of soil nitrogen pools.

Cooperating Organizations
California Coastal Commission
California State Coastal Conservancy
Caltrans
National Oceanic and Atmospheric Administration
U.S. Army Corps of Engineers
U.S. Fish and Wildlife Service

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Mitigating Loss of Eelgrass: Providing Sufficient Genetic Diversity

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R/CZ-108
1991–94

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The goal of this project is to evaluate the importance of maintaining sufficient levels of genetic diversity in transplanted eelgrass beds established for mitigation or restoration. The data from this project are the first available on reduction of genetic diversity in transplanted eelgrass beds relative to beds that have not been transplanted. Eelgrass transplantations for the most part are costly and not very successful despite well-established techniques and good protocols to select suitable sites. Insufficient genetic diversity may be a contributing cause to lack of success.

The first step toward achieving the objectives is to assess levels of genetic diversity in natural and transplanted eelgrass beds and to evaluate the spatial scale over which genetic structure (nonrandom distribution of genotypes or alleles) occurs. Once available, this information can be used to design an experiment to test the importance of genetic diversity for the development and success of transplantations. The following is a chronological description of the methods used to address the objectives, the results, and progress in meeting the objectives.

Genetic Diversity and Genetic Structure in Transplanted vs. Nontransplanted Eelgrass Beds

At least 72 eelgrass leaf shoots were collected from six eelgrass mitigation transplantations and from six beds that were not transplanted in San Diego County and San Quintin, Baja California. Enzymes were extracted from the leaves, and allozymes were separated by using starch gel electrophoresis. This process yielded six enzyme systems for a total of eight presumptive loci, of which four were polymorphic. Genetic diversity was assessed by using five indices: P, percentage of polymorphic loci; A, average number of alleles per locus; H_e, average frequency of heterozygotes expected under Hardy-Weinberg assumptions; H_o, observed frequency of heterozygotes; and G, proportion of distinct genotypes.

Mann-Whitney tests indicated that transplanted beds had significantly lower H_o, H_e, and G. The indices P and A were lower in transplanted beds, and this result approached conventional statistical significance ($P < .07$ for both indices). Plants from the sites in pristine San Quintin Bay had the greatest genetic diversity. Next in order were plants from southern San Diego Bay and the Silver Strand, which are the largest nontransplanted sites in San Diego County. The lowest genetic diversity, across all indices, occurred in plants from the transplanted sites on North Island and at Sail Bay. Half of the sites had no heterozygous individuals for the loci surveyed. Lower genetic diversity in plants in the transplanted sites was associated with smaller and younger eelgrass beds. Genetic diversity could be expected to increase over time because of migration of new genetic material into the beds via seeds, seedlings, and vegetative fragments that reestablish or through genetic recombination. However, no evidence indicated an increase in genetic diversity in transplanted beds sampled over 16 years, the age of the oldest transplanted bed sampled (Delta Beach; Table 1).

Genetic diversity likewise was not, as predicted, a positive function of the original size of the transplantations. Regressions of genetic diversity indices vs. size (log values) were not significant, although P and A were closer to a statistically significant relationship with size. All nonsignificant results were based on low power (Table 1).

Table 1. Regression Coefficients, Probability, and Power of Linear Regression Analysis of Genetic Diversity Indices vs. Size and Age of Transplanted Eelgrass Beds

Variables	*r*	P	Power
Log age vs:			
P	.00	.90	.03
A	.14	.80	.80
H_o	−.22	.68	.06
H_e	−.14	.79	.04
G	−.52	.29	.17
Log age vs:			
P	.73	.10	.36
A	.74	.09	.38
H_o	.00	.97	.03
H_e	.14	.80	.04
G	.63	.18	.25

Note: n = 6.

Spatial statistics (second-order analysis) were used to detect nonrandom distribution of rare alleles and genotypes at the two sites representing the most natural eelgrass (Silver Strand in San Diego Bay and False Bay, Baja California). Briefly, this analysis compares the distribution of alleles or genotypes sampled in a population to what the distribution would be if totally random in space (i.e., no genetic structure existed in the sample). The results of second-order analyses indicated generally little genetic structure at fine spatial scales (2–45 m). Of 46 analyses, only three indicated a nonrandom distribution of alleles or genotypes. This was the first analysis of the fine-spatial scale of genetic structure in eelgrass populations, and one of the first applications of this technique to marine populations in general.

Analyses of genetic structure did not provide evidence that eelgrass

populations in San Diego County are sufficiently different to treat each population as unique and thus to avoid selecting donor stock from beds not adjacent to the transplantation site. The purpose of this policy is probably to avoid adverse effects of disrupting locally adapted gene pools with unrelated ones (e.g., to avoid outbreeding depression). The hypothesis that outbreeding depression is an important consideration in eelgrass transplantation needs to be addressed experimentally. Results from the experimental transplantation (see following) did not support short-term outbreeding depression in eelgrass transplanted over 7 km within San Diego Bay.

Transplantation of Experimental Eelgrass

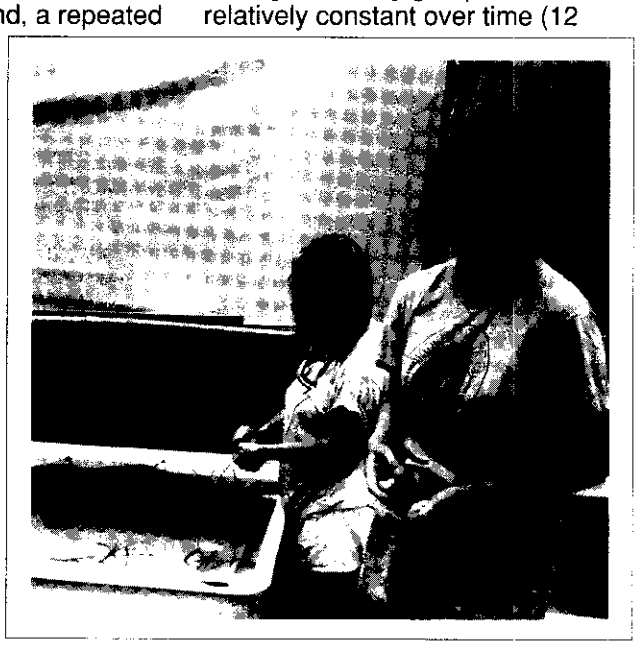
In August 1993, experimental transplantation of eelgrass at low and high genetic diversity was started at the Naval Command Control Ocean Systems Center on San Diego Bay. On the basis of the initial surveys of the genetic diversity of local eelgrass, the sites and necessary sample size were chosen to ensure a reasonable probability of collecting heterozygotes. After genotyping of 1300 individuals, plants were selected and placed in seven replicate plots of each treatment. All the low-diversity plots were planted with eelgrass of the most common genotype (fast allele at MDH and PGI loci). The high-diversity plots were planted with five MDH-PGI genotype combinations, including one individual of the rarest genotype found. The plants were collected from Silver Strand, the nontransplanted site in San Diego with the highest genetic diversity. Plots were 6.25 m^2. Each plot was planted with 40 transplant units; each unit consisted of one individual of known genotype. Transplant units were necessarily smaller (one intact rhizome with one to three leaf shoots) than those currently used in transplantations because of the necessity of knowing the genetic identity of each unit.

The density of leaf shoots was used as a measure of the success of the transplants. The numbers of flowering shoots and seedlings were also counted. At 5, 13, and 24 months after transplantation, 40 shoots in each plot were sampled randomly for genotyping and genetic diversity and structure analyses (only genetic diversity analyses are reported here).

Thus far, the transplantation as a whole has been successful. The original area of the transplantation (87.5 m^2) increased by 2.9 times, to 250 m^2 by June 1995 (22 months after transplantation), and the original mean leaf shoot density of $12 \pm 1/m^2$ (SD) increased to more than 300 during this period. The transplanted eelgrass grew significantly longer leaves (by 10 cm) than the donor population. New information on perceived problems with the transplantation protocol was discussed in the second-year report. For example, concerns about using donor eelgrass from only "adjacent" beds in San Diego are unfounded, and the rapid transplanting technique of using a single shoot (obviating labor-intensive packaging of bundles of eelgrass) apparently does not reduce eelgrass growth.

It is virtually impossible to assess the long-term effect of genetic diversity on successful transplantation of a long-lived species such as eelgrass under the constraints of a short-term research grant. Current mitigation policy requires a monitoring period of 5 years; we have been able to monitor for only 2 years. With this caveat in mind, a repeated measures analysis of variance of eelgrass leaf shoot density revealed a significant difference in the way the density changed over time between the low- and high-diversity plots. During the first 7 months, plants in the high-diversity groups grew more slowly on average because several plots were heavily grazed by brant geese. Thereafter, production of leaf shoots was higher in plants in the high-diversity groups than in the low-diversity group (Figure 1). At the end of this project (22 months after transplantation), the average rate of increase in leaf shoots per month was 17 ± 11 in the low-diversity plants and 26 ± 10 in the high-diversity plants. Although this result may suggest that genetic diversity could have a beneficial effect on short-term success of transplantation, it is too early to form conclusions, and the result is based on a small sample size ($n = 7$ of each diversity level).

Probably more useful is the information on how genetic diversity changes in eelgrass transplantations over time. In addition to providing information useful for designing eelgrass beds for mitigation and restoration projects, the experimental transplantation is the first to provide empirical evidence of temporal changes in genetic diversity over time in small eelgrass populations. The genetic diversity in the high-diversity groups remained relatively constant over time (12



Preparing eelgrass to genotype for transplanting.

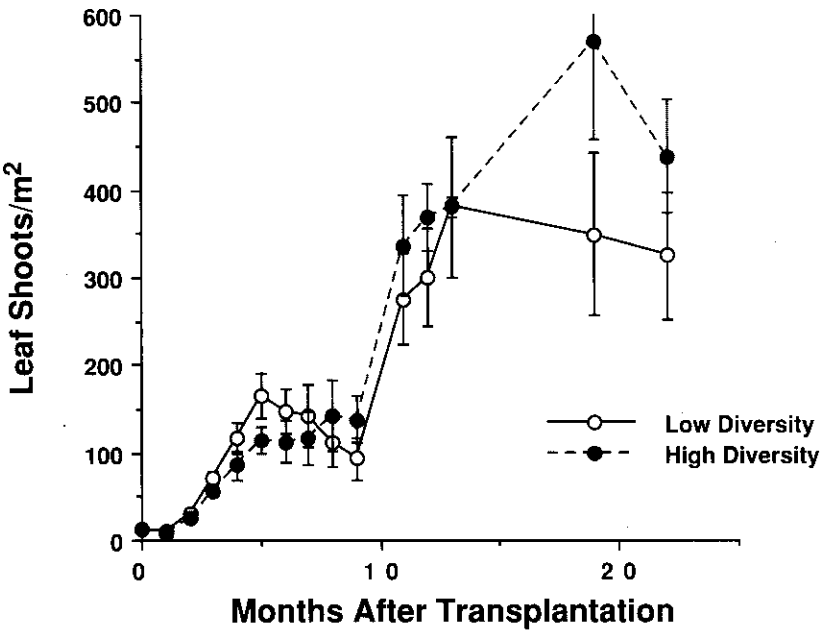


Figure 1. Short-term success of eelgrass planted at high and low levels of genetic diversity. n = 7. Data are means ± standard error.

months), whereas that in the low-diversity group fluctuated more. This result was statistically significant. The average percentage of polymorphic loci in the high-diversity plants was 86% of the original at the end of 13 months, whereas that of the low-diversity group dropped to 17% of the original. We are completing analysis of samples obtained 2 years after transplantation. Interestingly, a higher proportion of heterozygous individuals flowered and produced seeds than in the population as a whole.

Summary

Transplantation of eelgrass for mitigation in San Diego County has resulted in a significant loss of genetic diversity (up to 80%). Furthermore, urban marine eelgrass beds in San Diego County that were not transplanted are low in genetic diversity compared with pristine beds in nearby Baja California. No evidence was found that genetic diversity in transplanted beds increases naturally over time, at least up to 16 years, and overall genetic diversity declined in the eelgrass transplanted in this project, despite sexual reproduction by the transplants.

Environmental managers confronted with eelgrass restoration repeatedly question the importance of genetic diversity, particularly in the face of rapid deterioration and destruction of habitat. The argument that the changes in habitat have greater short-term importance to eelgrass persistence than genetic effects do echoes a general sentiment among some scientists. Unfortunately, lack of data restricts this argument to a debate, because only a few theoretical and empirical analyses address the relative importance of demographic, genetic, and environmental factors to the persistence of populations. Recent studies have shown the artificiality of separating demographic, genetic, and environmental effects (e.g., Lande, 1994). According to theoretical and limited empirical data, genetic diversity is important for the long-term persistence of populations. Defining long term, however, remains a critical problem for clonal plant biologists. A more tractable question concerns the rapidity of environmental change. For example, rise in sea level and coastal erosion are predicted to raise the tide line by 1m in San Diego by the year 2010. Because the natural, gently sloping shorelines around many bays in Southern California have been replaced by stone revetments, no high intertidal refuge from increasing water depth and reduced light now exists for eelgrass, which tends to be very light limited. If eelgrass has insufficient genetic diversity to adapt to reduced light as sea level rises, its distribution likely will decrease. Therefore, beds with relatively high genetic diversity, such as the bed at Silver Strand in San Diego and the pristine beds in Mexico, should be given a high priority for conservation.

Cooperating Organizations

National Marine Fisheries Service, Terminal Island
California Department of Fish and Game
Pacific Southwest Biological Services
MBC Applied Environmental Sciences
U.S. Navy, Navy Facilities Engineering Command, Naval Command Control Ocean Systems Center
Centro de Investigaciones Cientifica y de Educacion Superior de Ensenada
City of San Diego

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Lectures and Conferences

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Williams, S.L. Seagrasses and the sustainable biosphere initiative. Invited workshop leader, Estuarine Research Federation Meeting, Hilton Head, North Carolina, November 1993.

Williams, S.L. Genetic diversity of seagrasses and seaweeds: The management challenge. One of six invited papers, Symposium on Conservation of Biodiversity Within Coastal Environments, Western Society of Naturalists Meeting, Los Angeles, January 1994.

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Seismic Risk of the Offshore San Gregorio–Hosgri Fault Zone Between Monterey and San Francisco

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R/CZ-110
1991–93

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Large earthquakes along offshore faults pose a potential hazard to coastal California cities. After the 1989 Loma Prieta earthquake, the U.S. Geological Survey increased probability estimates of a major earthquake during the next 30 years along several faults in the San Francisco Bay area, all of which are part of the San Andreas fault system marking the diffuse boundary between the North American and Pacific plates. This risk is further compounded by the fact that any earthquake of a magnitude greater than 7 within 100 km of San Francisco may cause damage in the areas underlain by unconsolidated soils (Holzer, 1994).

The primary objective of this project was to test the hypothesis that the offshore member of the San Andreas fault system in the Bay area, the San Gregorio fault, is the site of recent thrust (reverse) movement, which could produce significant vertical ground accelerations during a major earthquake. This hypothesis is based on the growing recognition of active thrust and oblique-slip faulting elsewhere along the central California continental margin (McCulloch, 1987), including the Hosgri fault, which probably forms the southern continuation of the San Gregorio system (Graham and Dickinson, 1978). Slip direction along the San Gregorio fault has been interpreted as: (1) right lateral strike-slip (Greene, 1977; Graham and Dickinson, 1978), (2) thrust (Crouch et al., 1984), and (3) a combination of high-angle reverse and strike-slip (Greene, 1990). The change from strike-slip to oblique and thrust movements along many offshore faults has been cited as a response to changes in motion of the Pacific and North American plates between 3 million and 4 million years ago (McCulloch, 1987). If recent thrust (reverse) movement is confirmed, then coseismic deformation along the San Gregorio fault may pose a greater seismic hazard than previously believed because of its potential to (1) produce significant vertical ground accelerations and (2) generate local tsunamis.

Previous Studies

In 1926 the San Gregorio fault, which crosses Monterey Bay, was the probable site of two Monterey Bay earthquakes with estimated magnitudes of 6 (Gawthrop, 1978). Several studies have concluded that the San Gregorio fault is capable of generating an earthquake of magnitude 7 or greater (see Tuttle, 1985). One method of estimating the potential magnitude of an earthquake along active faults depends on the predicted length of fault rupture, which is, in some cases, controlled by the length of fault segments. This parameter is currently poorly constrained for the San Gregorio fault. South of the Monterey Bay, the San Gregorio fault is generally thought to connect with the Hosgri fault (Graham and Dickinson, 1978), creating a system fault more than 400 km long that has been the object of scrutiny because of its proximity to the Diablo Canyon Nuclear Power Plant. However, Greene (1977, 1990) projects the San Gregorio onshore north of Point Sur along the Palo Colorado fault, significantly modifying estimates of fault length.

North of Monterey Bay, the San Gregorio fault extends onland at Point Año Nuevo but returns to the offshore area near San Gregorio Beach, where it projects toward the southern end of the Seal Cove fault at Pillar Point. The nature of the connection with the Seal Cove fault is, however, poorly understood.

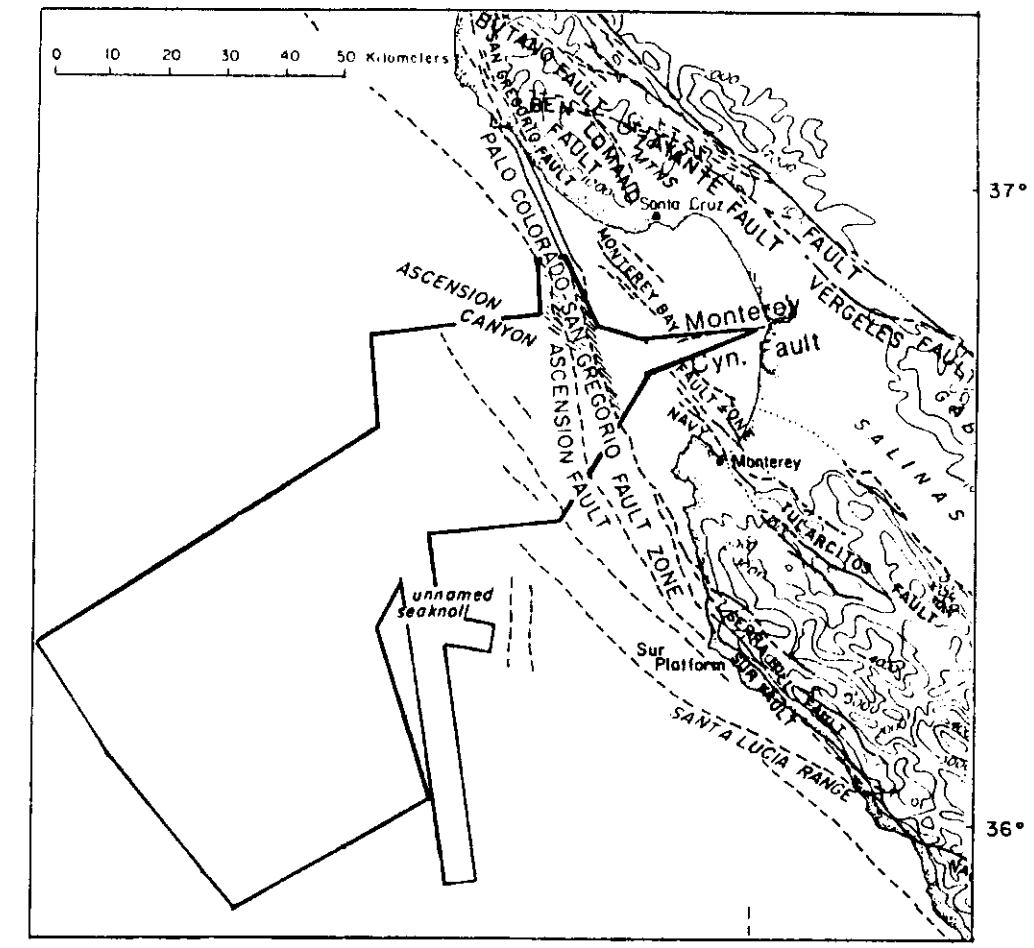
Estimates of modern slip rate, which included analyses of seismic risk, depend on the amount of fault offset. Estimates of late Cenozoic displacement along the San Gregorio fault range from less than 10 km to as much as 150 km (Hall et al., 1995). Slip rates estimated for the San Gregorio-Hosgri system vary between 0 and more than 12 mm/year (Anderson et al., 1990).

Research Program

This program consisted of four complementary studies: (1) mapping of the seafloor along the offshore segment of the San Gregorio fault, (2) submersible expeditions, (3) analyses of offshore earthquakes after the 1989 Loma Prieta earthquake, and (4) field studies of marine terraces and coastal outcrops of the San Gregorio and Seal Cove faults between San Gregorio Beach and Montara State Beach.

Seafloor mapping. The seafloor mapping program was based on a multifrequency side-looking sonar survey of the Monterey submarine canyon directed by W. Ryan of Columbia University in September 1990 (Figure 1). Project Leader Reed supervised the portion of the survey along the San Gregorio fault. Processing and digital registration of sonar images to gridded SeaBEAM bathymetric data were performed, and the data sets were merged into a mosaic image showing the nature of seafloor acoustic backscattering and morphology, which was used to map the distribution of recent deformation and mass wasting along the fault (Figure 2).

Diving program. The submersible program consisted of (1) an expedition using the deep sea vessel *Sea Cliff* of the U.S. Navy in 1991, which was provided by the Monterey Bay Aquarium Research Institute, and (2) high-resolution video images and seafloor samples



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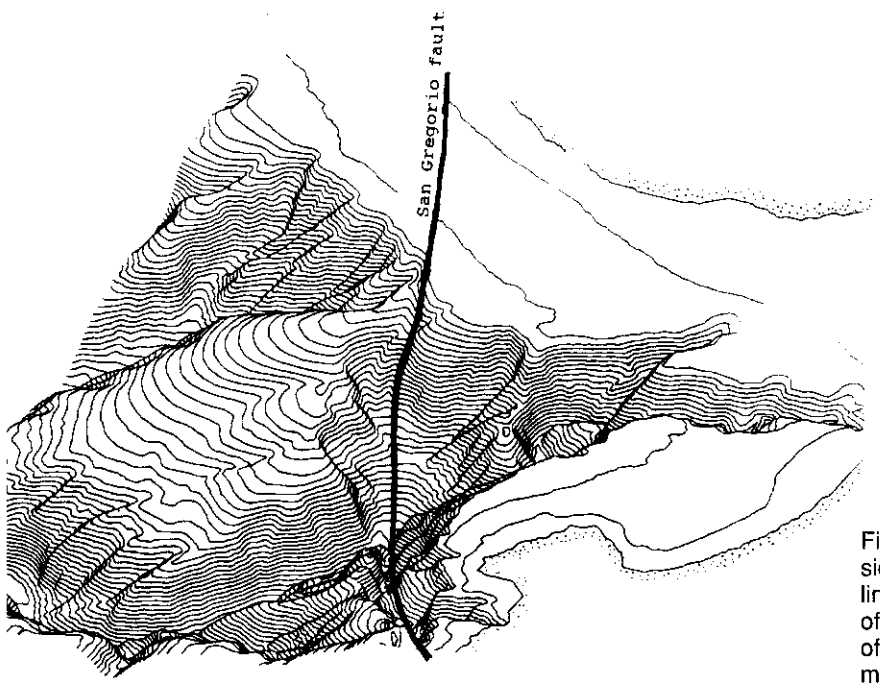

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Figure 1A, Outline of multifrequency side-scan sonar survey with diagonal lines showing region of detailed survey of the San Gregorzio fault and location of submersible dives; base map modified from Greene, 1990. B, Perspective view of seafloor morphology looking to the northwest along the San Gregorio fault and across the Monterey canyon.

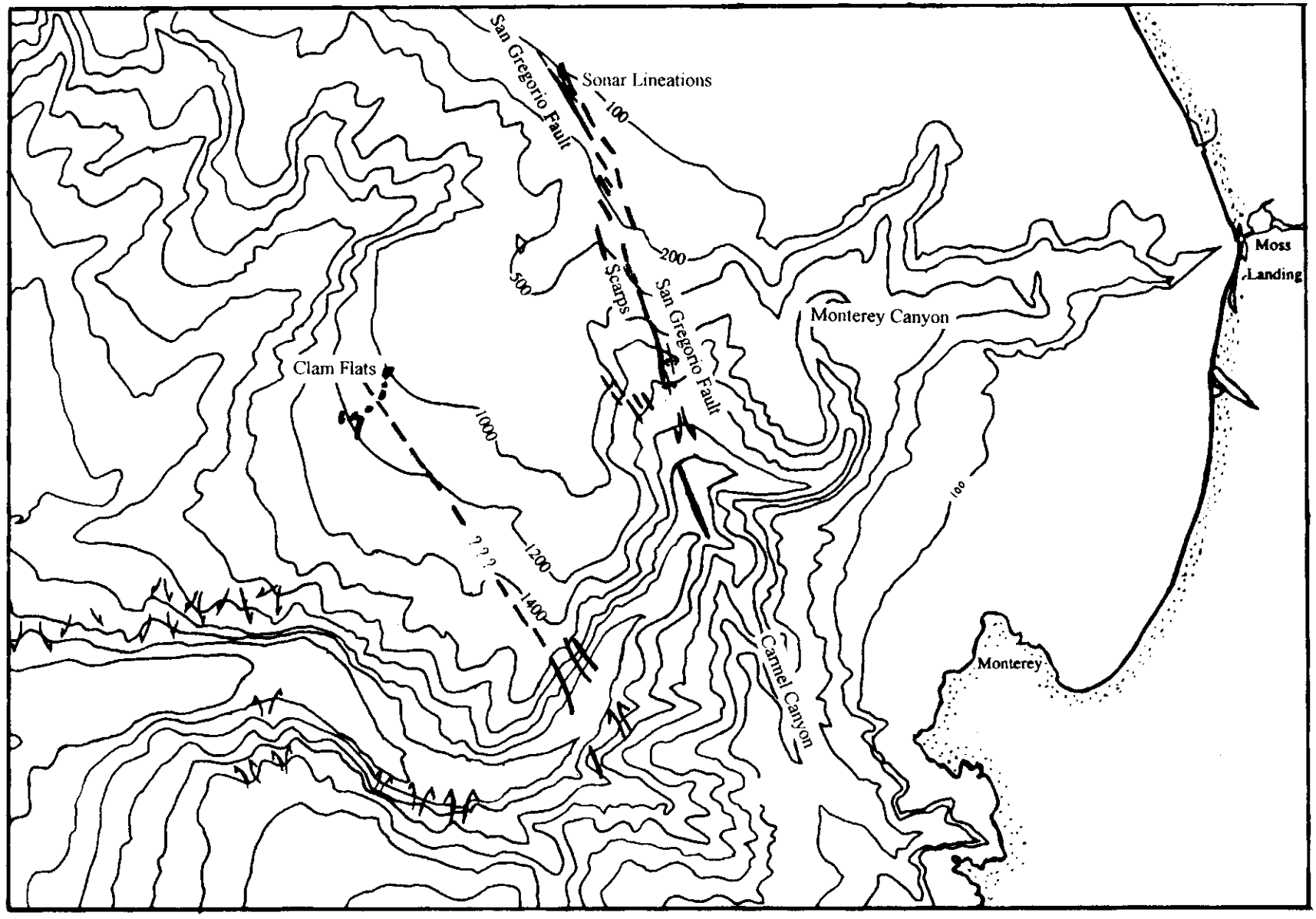

Figure 2. Map of locations of seafloor deformation along the San Gregorio fault.

acquired with the remotely operated vehicle *Ventana* of the Monterey Bay Aquarium Research Institute in 1993, in collaboration with Daniel Orange. The combination of dives provided considerable information for verifying the sonar image.

Offshore earthquakes. David Oppenheimer of the U.S. Geological Survey provided relocations and focal mechanisms of offshore earthquakes, which followed the 1989 Loma Prieta earthquake. Project Leader Reed joined in the interpretation of these earthquakes and integration of these results with the seafloor mapping program.

Coastal field studies. The program of coastal field studies was moved northward from the Point Sur region to the area between San Gregorio Beach and Moss Beach in order to study fault segmentation between Pillar Point and San Gregorio. Marine terrace elevations and coastal outcrops were examined between Point Año Nuevo and Moss Beach along with San Gregorio and Seal Cove faults.

Results

The San Gregorio fault system is marked on the sonar image by several parallel lineations, which cross the axis of the Monterey submarine canyon and extend northwestward onto the continental shelf near Point Año Nuevo (Figure 3). These features are discontinuous but typically lie within a region 2–3 km wide that has several seafloor scarps and anticlinal ridges. One of the scarps was observed with the remotely operated vessel *Ventana*, which revealed a feature 2-m high that had several subsidiary joint and fault systems (Figure 4). A set of small steps was observed on the scarp face suggesting vertical slip on a fault with uplift on the west side of the fault. This interpretation is, however, inconsistent with interpretations of multichannel seismic profiles (Greene, 1990) showing uplift of rock units on the east side of the fault. Seafloor outcrops of consolidated strata were sampled during the *Ventana* expedition. Samples on both sides of the San Gregorio fault are virtually indistinguishable from each other

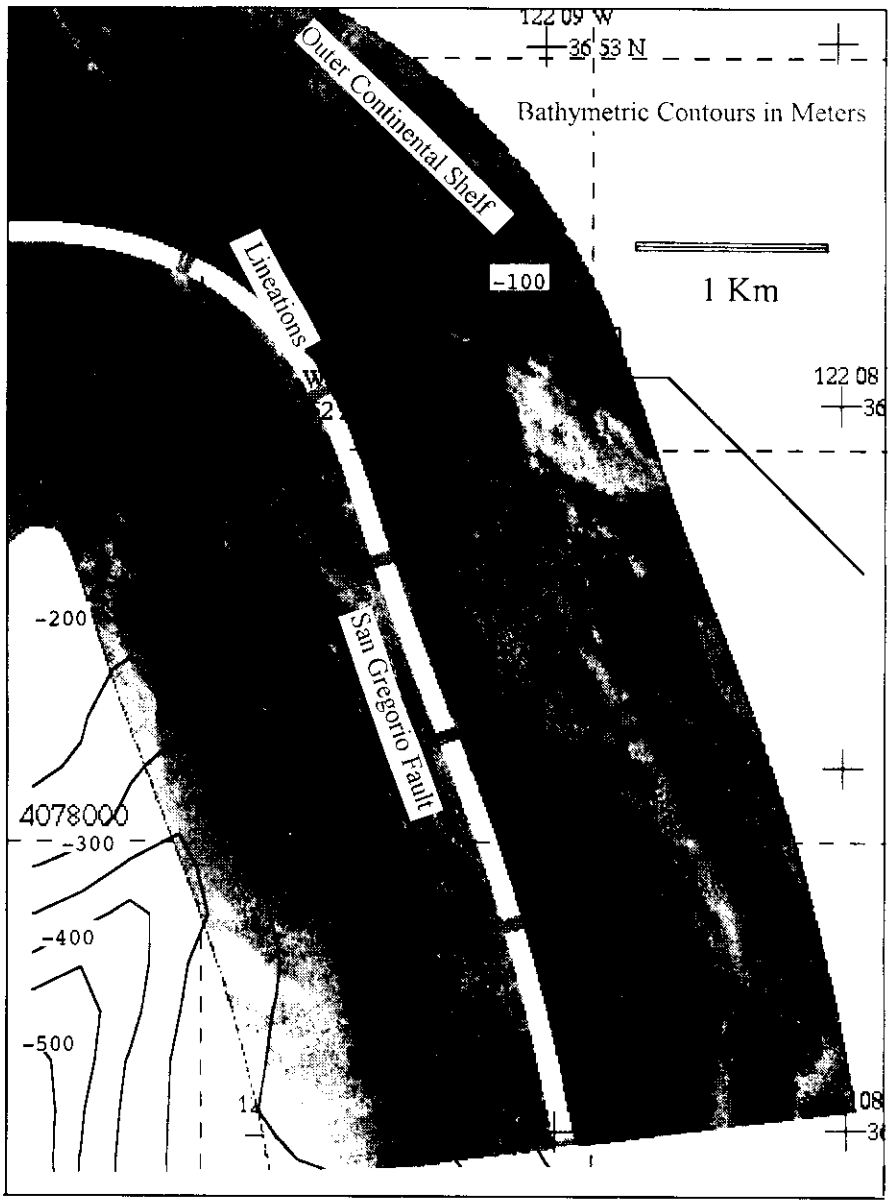

Figure 3. Side-scan sonar image with coregistered SeaBEAM bathymetry of the seafloor deformation and carbonate deposits along San Gregorio fault along outer continental shelf south of Point Año Nuevo.

and from the Purisima formation, which has been mapped along the coast (Clark et al., 1984) and in the offshore (Greene, 1977), suggesting that these rocks provide little evidence of significant post-Pliocene lateral offset.

The fault is marked by several distinctive lineations in the sonar image along the outer continental shelf south of Point Año Nuevo, which result from diagenetic carbonate deposits, produced from the seepage of methane from the seafloor, and large populations of brachiopods (Orange et al., 1993). Mass wasting along the fault is common, and submarine erosion along the fault controls the orientations of canyon heads near the shelf break in the northern part of Monterey Bay.

Discontinuous sonar backscattering along the fault near the northern slope of the canyon at a depth of 1450 m was examined with the deep sea vessel *SeaCliff* and appears to reflect a blanket of mud, which covers several low-relief ridges. The latter features may be related to

Figure 4. Side-scan sonar image with coregistered SeaBEAM bathymetry of the seafloor scarp along San Gregorio fault south of Point Año Nuevo.

pressure ridges developed by compressional stress normal to the San Gregorio fault zone.

Many seafloor features are parallel to the general trend of the fault zone, including small ridges that we interpret as anticlinal fold axes, a finding in marked contrast to that predicted by models of deformation associated with strike-slip faults. On the other hand, Dr. Orange has mapped north-south vein-filled fractures along the fault zone on the outer continental shelf, suggestive of active strike-slip faulting. The trace of the fault system is linear over the irregular seafloor topography, indicating a relatively steep-dipping structure at least in the upper 2 km of the crust, consistent with interpretations of others (Greene, 1990; Lewis, 1990).

The number of offshore earthquakes per month increased markedly immediately after the 1989 Loma Prieta earthquake but has decreased to a rate that is considered normal on the basis of historical seismicity. Reasenberg and Simpson (1992) attributed increased rates of seismicity along the fault, particularly near Point Año Nuevo, to increased static stress along the fault triggered by the 1989 Loma Prieta earthquake. Sebrier et al. (1992) concluded from studies of Quaternary structures onshore and focal mechanisms of offshore earthquakes that the region between the San Gregorio and San Andreas faults is undergoing fault-normal compression. Our analyses of offshore earthquakes that occurred after 1989 show a large number of oblique-slip events along the San Gregorio fault, which include both right-lateral strike-slip and reverse components, consistent with the combination of vertical and lateral fault displacements observed along offshore faults. Surprisingly, most of the recent earthquakes in the southern part of Monterey Bay were located several kilometers to the east of the San Gregorio fault. These locations cannot be ascribed to mislocation errors according to the U.S. Geological Survey. Consequently, trace of recent seismicity in southern Monterey Bay projects onto the Monterey Peninsula in the vicinity of the Cypress Point fault and other faults to the east. This result may require a revision of estimates of seismic risk as well as estimates of the length of active faults in the region, including the San Gregorio fault.

The Half Moon Bay terrace, dated at 70,000–120,000 years before the present, forms a broad anticlinal feature between Half Moon Bay and San Gregorio State Beach (LaJoie et al., 1979). The magnitude of uplift correlates closely with the proximity of the San Gregorio fault and falls off with distance away from the fault. Anderson and Menking (1994) modeled uplift of Quaternary terraces along the northern Santa Cruz coastline from which they suggested a vertical slip rate of 0.5–0.9 mm/year along the San Gregorio fault. Dextral slip rates are poorly constrained by their models but appear to be significantly higher.

Farther north, near the Half Moon Bay airport, the Half Moon Bay terrace is offset as much as 45 m vertically along the Seal Cove fault, with uplift along the west side of the fault (LaJoie et al., 1979). Crustal movements along the Seal Cove fault therefore include a significant amount of vertical displacement, as well as strike-slip faulting, which has been emphasized by earlier studies. In contrast, exposures of the San Gregorio fault between Point Año Nuevo and San Gregorio State Beach show uplift of rocks along the east side of the fault, and terrace deformation in the latter region is distributed over a wide region.

In addition to a change in the sense of movement, the Seal Cove fault system steps to the west of the San Gregorio system at Pillar Point. Rates of recent seismicity also differ between the two fault segments, with significantly lower rates on the Seal Cove fault (Tuttle, 1985). Consequently, a fundamental break, possibly corresponding to termination of San Gregorio fault segment, may be located between San Gregorio Beach and Pillar Point.

Summary and Conclusions

The San Gregorio fault in the northern part of Monterey Bay shows evidence of recent oblique slip characterized by both dextral strike-slip and reverse (thrust) slip. Consequently, vertical as well as horizontal ground motions may occur during coseismic deformation, especially in regions of soft soil. Several characteristics of the Seal Cove and San Gregorio faults differ greatly, suggesting fault segmentation between San Gregorio Beach and Pillar Point. This observation may require a downward revision in estimates of the length of the San Gregorio fault, which include the Seal Cove fault, and potential earthquake magnitudes in the region. In the southern part of Monterey Bay, the highest rate of seismicity is located to the east of the San Gregorio fault and projects onshore at the Monterey Peninsula. Each of these conclusions should be incorporated into estimates of earthquake risk to coastal communities in the region.

Cooperating Organizations

Crew of research vessel *Laney Chouest* and deep sea vessel *SeaCliff*
Crew of research vessel *Point Lobos*
Crew of research vessel *Point Sur*
Institute of Marine Studies, University of California, Santa Cruz
Lamont-Doherty Earth Observatory of Columbia University
Monterey Bay Aquarium Research Institute
San Jose State University
U.S. Geological Survey

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Natural Time Scales of Variability in Coastal Pelagic Fish Populations of the California Current Over the Past 1500 Years: Response to Global Climate Change and Biological Interaction

Scripps Institution of Oceanography, UCSD
R/CZ-112
1992–95

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Introduction

Management of the Pacific sardine and northern anchovy stocks off California has been hampered by uncertainties stemming from poor understanding of the expansions and contractions of these populations occurring over several decades. Because of the difficulty in sampling and documenting these scales of variability, the CalCOFI program developed a strategy based on the study of accumulation rates of fish scales in bottom sediments (Soutar and Isaacs, 1969, 1974). Reconstruction of the fish scale-deposition rates provides historical perspective on the range and nature of past variability and a frame of reference in which to place modern observations (Baumgartner et al., 1992). This research is possible because of the good preservation of fish scales within a chronological framework of annually deposited layers in the enclosed Santa Barbara Basin where the bottom waters are depleted in oxygen and sedimentation rates are high (Soutar and Crill, 1977).

A principal goal of this project has been to validate and refine the original reconstructions of the variability in the sardine and anchovy populations over the past 1500 years which were published by Baumgartner et al. (1992). This included doubling the resolution of sampling and insuring adequate replication of the observations. Achieving this goal required a significant effort towards constructing a detailed chronological framework, first by identifying and counting the annual layers (known as "varves") from multiple core sites and then by evaluating this chronology using an independent method of dating. A major achievement of this project has been to initiate development of the application of radiocarbon measurements as this independent source of dating and verification. The other major focus of this project has been developing the use of background environmental information, independent of the fish scale analyses, to reconstruct changes in ocean climate which are associated with variation in the fish populations.

Collection and Processing of Sediment Cores

During the period of our previous (1991–93) project we collected two Kasten cores and two short Soutar-type box cores from the Santa Barbara Basin. In the project period reported here, we collected three additional Kasten cores and two box cores (Table 1). KC-9110-1301 was opened, slabbed, and X-radiographed for the previous project. Two Kasten cores (9110-1302 and 9210-1001) were opened, slabbed, and X-radiographed during this project. The radiography maps the internal structure from which a composite chronostratigraphy is developed by identifying and counting the varves. The sediment columns from the cores were sliced into six slabs. Two slabs of 1-cm thickness were taken for detailed, multiple X-radiography of the fine structure to reveal the stratigraphy down the core. Four more slabs of 2.5-cm thickness were taken for subsampling of the fish scales and other components of interest. The radiography was carried out by J. Slovecek with assistance from T. Knower. V. Ferreira was in charge of slabbing, preliminary chronostratigraphy, and subsampling of the cores.

Table 1. Kasten and Soutar-box Cores Collected from the Santa Barbara Basin October 1991 through August 1993

Kasten Cores

Designation	Location	Depth	Length	X-Radiographed
KC- SB-9110-1301	34°–12.0′N, 120°–00.2′W	588 m	258 cm	Total
KC- SB-9110-1302	34°–12.7′N, 120°–03.4′W	588 m	268 cm	Total
KC- SB-9210-1001	34°–13.4′N, 120°–01.6′W	590 m	266 cm	Total
KC- SB-9210-1002	34°–13.6′N, 120°–05.8′W	585 m	237 cm	
KC- SB-9308-2301	34°–13.3′N, 120°–03.8′W	590 m	277 cm	

Box Cores

Designation	Location	Depth	Length	X-Radiographed
BC- SB-9110-1301	34°–12.9′N, 120°–00.7′W	585 m	1	1
BC- SB-9110-1302	34°–12.9′N, 120°–03.2′W	588 m	1, 2	1, 2
BC- SB-9210-1001	34°–13.3′N, 120°–01.7′W	588 m	1, 2, 3	2
BC- SB-9210-1002	34°–13.8′N, 120°–06.1′W	580 m	1, 2	

Note: Table indicates the location and bottom depth of core sites plus core length (for Kasten cores) and progress in opening and X-radiography of cores. The sample designation indicates the type of core, the location, and the year, month, day, and sequence of retrieval of cores on a particular day—i.e., KC-SB-9110-1301 is a Kasten core recovered from Santa Barbara Basin on October 13 1991, and was the first core recovered on that date. The Kasten corer has a 305-cm-long barrel with a fitted acrylic liner with inside dimensions of 14 × 14.3 cm. The Soutar-type box cores are collected in conjunction with the Kasten cores in order to obtain an undisturbed surface with the upper 80 to 100 years intact. The box core material was recovered as subcores by acrylic boxes with inside dimensions of 10 × 11.5 cm.

Figure 1 indicates the detailed laminar structure of the three Kasten cores, providing an overview of the stratigraphic relationships among the cores. The fine stratigraphy is produced by continuous deposition and preservation of seasonal laminae couplets year after year. The correlation lines drawn between the cores mark the upper and lower boundaries of major turbidites resulting from gravity flows originating on the basin slopes. The turbidites represent instantaneous events of deposition and are therefore not counted in the compilation of the chronostratigraphy.

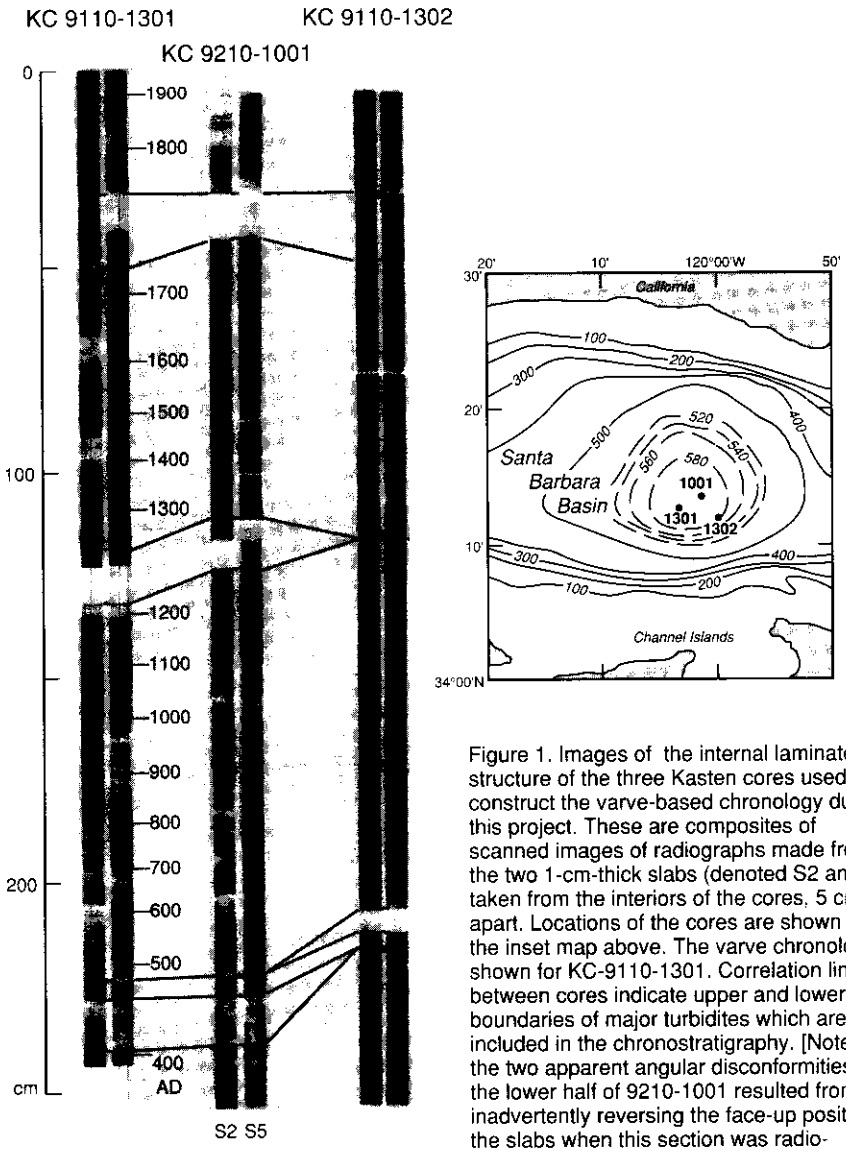

Figure 1. Images of the internal laminated structure of the three Kasten cores used to construct the varve-based chronology during this project. These are composites of scanned images of radiographs made from the two 1-cm-thick slabs (denoted S2 and S5) taken from the interiors of the cores, 5 cm apart. Locations of the cores are shown on the inset map above. The varve chronology is shown for KC-9110-1301. Correlation lines between cores indicate upper and lower boundaries of major turbidites which are not included in the chronostratigraphy. [Note that the two apparent angular disconformities in the lower half of 9210-1001 resulted from inadvertently reversing the face-up position of the slabs when this section was radiographed.]

Development and Validation of Chronology

An accurate chronostratigraphy is essential to developing reliable historical reconstructions of fish populations and background environmental information. We began this project with a completed preliminary varve count in Kasten core KC-9110-1301 which assigned calendar years to the varve counts. This was anchored at the top by correlation to chronology of the recent period back to 1870 AD established earlier by cross-correlation among five box cores, two of which were dated with ^{210}Pb and $^{238}Th/^{232}Th$ (Soutar and Isaacs, 1974; Soutar manuscript in preparation).

This preliminary chronology was used as the starting point for developing a refined varve chronology by examination and cross-checking among all three of the Kasten cores. The improved chronology is indicated on Figure 1 in relation to the stratigraphy of KC-9110-1301. Although we usually find good and often excellent varve preservation in these sediments, the quality of preservation is occasionally degraded by episodes of bioturbation, resulting in minor disruptions of laminae to erasure of the varve structure. Accumulation of these episodes produces an inherent uncertainty which can be reduced to a large degree by meticulous examination and correlation of the stratigraphy across the three cores. However, judging the accuracy of the resulting chronology (and any marine varve chronology through the period of the past several thousand years) is limited by the lack of techniques developed for radiometric dating. This led us to propose to work on an application of AMS radiocarbon dating to provide independent ages from the sediments of the past 2000 years. To undertake this work we developed a collaborative effort with J. Southon of Lawrence Livermore National Laboratory to explore the use of radiocarbon measurements from biogenic ocean carbonate secreted in near surface waters using the pelagic pteropod *Limacina helicina* (generally found to be concentrated in the upper 100 m of the ocean; C. Lalli, University of British Columbia, personal communication). We now have a nearly continuous record for the period from 1920 AD back to 400 AD, consisting of more than 130 AMS ^{14}C dates from *L. helicina*, with sample intervals varying from 5 to 20 years in length. Picking and initial preparation of the *L. helicina* samples was carried out by J. Field.

When this sequence of radiocarbon ages (not shown here) is assigned calendar dates derived from the varve chronology in Figure 1 we can obtain a corresponding series of ocean-terrestrial age differences (the ocean-reservoir ages, also not shown). These are

the radiocarbon age differences between the *L. helicina* series and the time series of bidecadal wood dates calibrated to calendar years by tree ring chronologies (Stuiver and Pearson, 1993). Then, calculation of expected radiocarbon activities in a simple ocean model (Stuiver and Braziunas, 1993) provides a unique signature through time which can be used as a reference for comparison to the ocean-reservoir ages. Our detailed series of radiocarbon dates permits us to recognize this idealized reference signal within the variability of the *Limacina* ages and can therefore be used as an independent means to judge the accuracy of our varve chronology. We are now refining the technique so that comparison of the variability in *Limacina*-wood radiocarbon differences with the model allows us to correct the overall chronology of the past 1500 years with an uncertainty approaching ±10 years. Application to the current reconstruction of sardine and anchovy series has resulted in major improvements to documenting the time scales of variabililty in the populations (see results below).

Reconstruction of Sardine and Anchovy Variability

We have completed the fish scale counts from the four thick slabs of Kasten core 9110-1301 with a sample resolution of 5 years, adjusted to the improved chronology shown in Figure 1. The fish scale counts were carried out by J. Field. In addition, we have completed 90% of the fish scale counts needed for development of a similar set of four SDR series from KC 9110-1302. We will soon extend the fish scale sampling and counting to KC 9210-1002 with support from the National Science Foundation Earth System History program.

An important goal of this project was to examine the reproducibility of the fish scale deposition signals in the Santa Barbara Basin by comparison of the series developed here and the original series developed by Soutar from two small diameter piston cores (Baumgartner et al., 1992). This comparison is made in Figure 2 with the two sets of sardine and anchovy series. The higher sampling frequency in KC 9110-1301 results in a noisier appearance and greater "spikiness" compared to the original series, which is smoothed as a result of the lower resolution of 10-year intervals. The most important difference between these two pairs of series, however, is in the chronologies. Comparison of the time axes shows a difference of nearly 100 years at the point at which they begin. This offset gradually diminishes upcore and is reconciled by 1815 AD by splicing both series to the same data set developed earlier by Soutar and Isaacs (1974) using a 5-year sampling resolution in four box cores. The striking difference of an entire century in Figure 2 emphasizes the need for a robust independent means to verify and adjust the varve chronology.

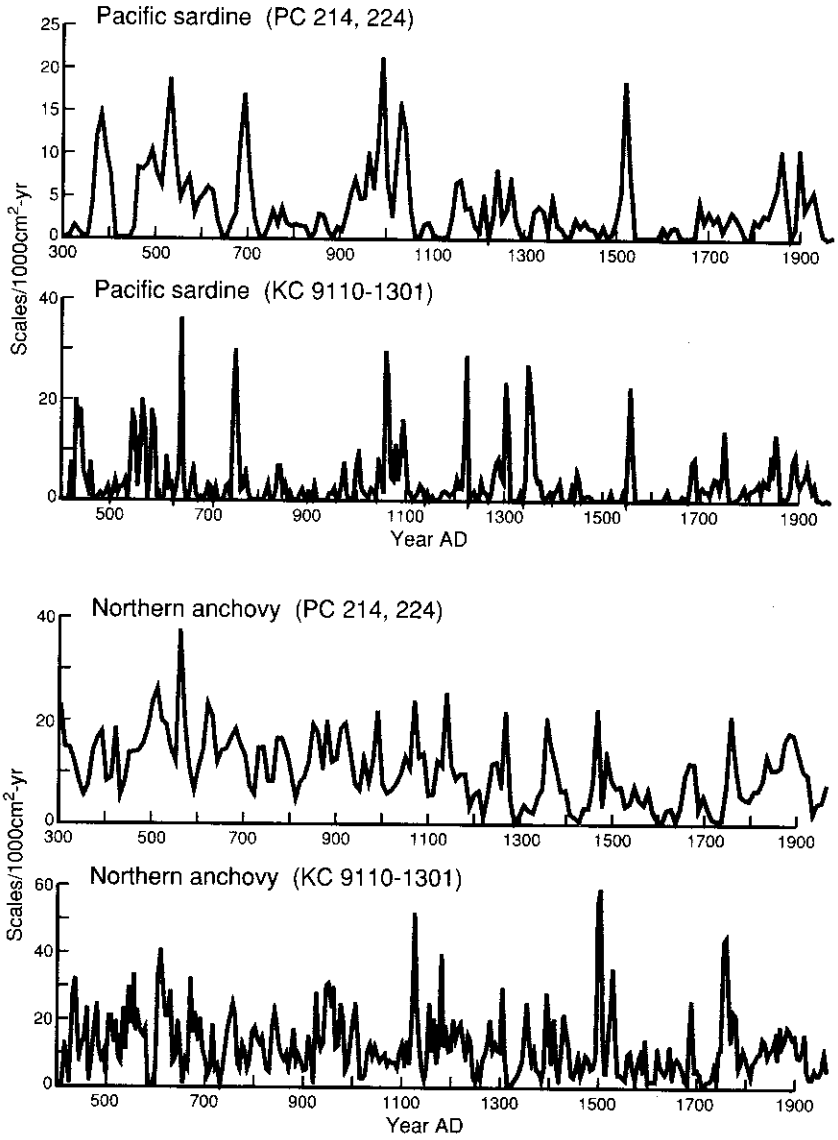

Figure 2. Comparison of the series of the sardine and anchovy scale-deposition rates developed during this project (KC 9110-1301) and the original series from Baumgartner et al. (1992). Counts for the original series were made with samples taken from sequential 1-cm slices down two 7.6-cm diameter piston cores (PC 214, PC 224). These were adjusted to represent 10-year intervals after development of a chronology based on varve counts. Values in the original series are averages between the two piston cores. Values on the new series are averages from four longitudinal slabs down KC 9110-1301. The new series is based on a sampling resolution of 5 years. The two piston cores represent a small sample of depositional area relative to the Kasten core. One of the Kasten core slabs provides a sample of depositional area which is roughly three times greater than that given by one of the piston cores. The total depositional area sampled by the Kasten core four slabs is slightly greater than 130 cm².

Despite the chronological offset and differences in resolution, the same fundamental structure of variability is contained in both pairs of series—noteworthy because these series were developed completely independent from one another—confirming that the scale-deposition rates (SDRs) over the Santa Barbara Basin are indeed reproducible. Completion of the SDR series from the remaining two Kasten cores will provide very robust time series of sardine, anchovy, and hake populations in the California Current.

Calibration of the SDRs as indices for the sardine and anchovy biomass has also been improved during this project in comparison to that provided by Baumgartner et al. (1992; see their Figure 6). This was achieved by first transforming the SDR data to natural logarithms before calculation of the regression (not shown here) with modern population estimates. Using this relationship, we have made a new hindcast of sardine and anchovy biomasses for the central region of the California Current. Figure 3 shows these hindcast biomasses separated into distinct modes of variability based on the characteristic frequencies comprising the variance. Figure 3a is a plot of the high-frequency components composed of periods shorter than 100 years. Figure 3b shows the low-frequency components of the two species and indicates the very-low frequency change in both species by the long-term trendlines. Dissection of the variance into these frequency components allows us to describe the characteristic time scales of variability—and to make a further comparison to the analysis by Baumgartner et al. (1992). Because of the relative compression of the new time series apparent in Figure 2, we find that the main peaks in the variance spectra (see Figure 4) have been shifted considerably with respect to the analyses of the original series (Baumgartner et al., 1992; compare Figure 4 with their Figures 9 and 10).

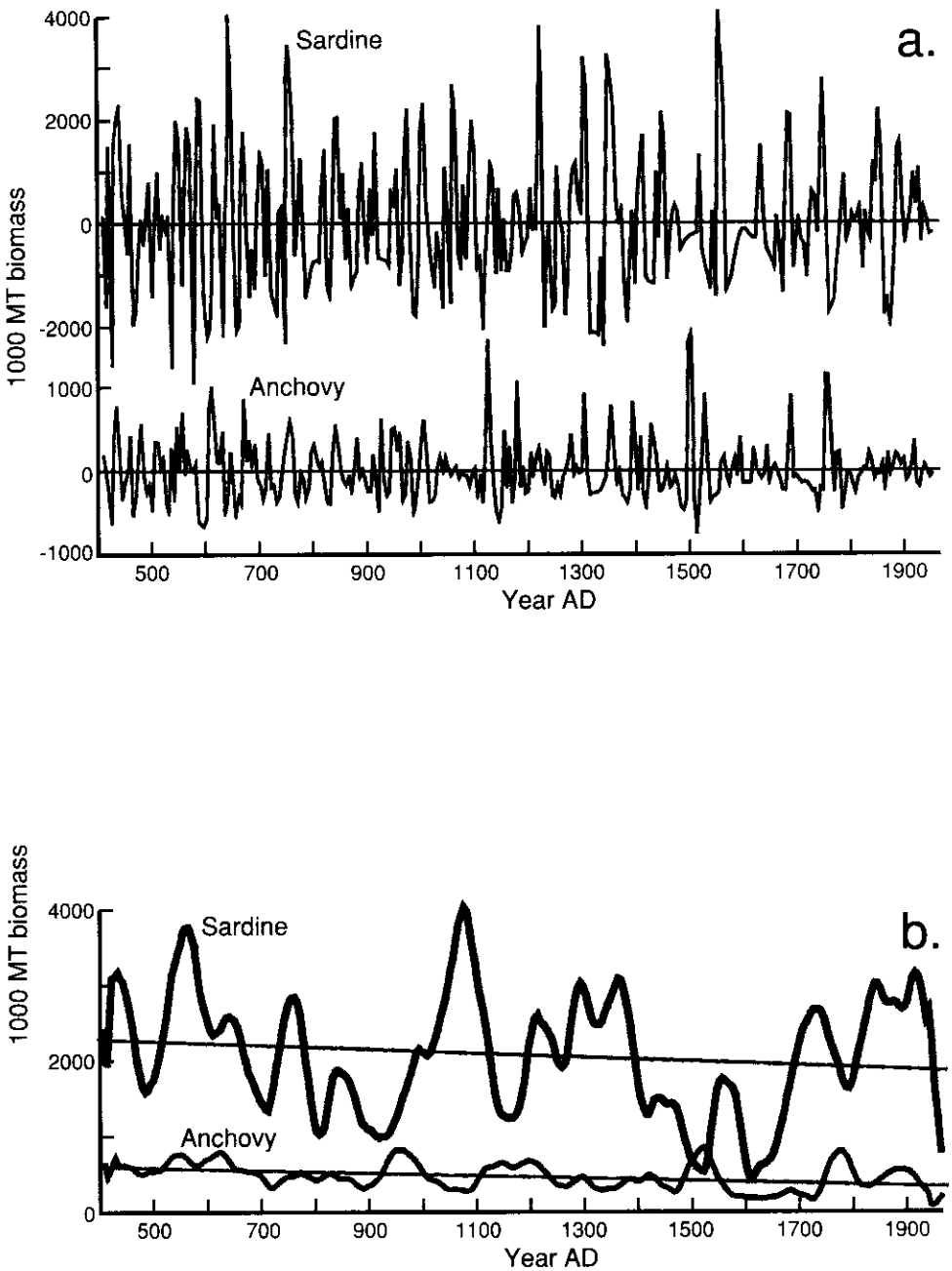

Figure 3. Time series of the hindcast biomass for the sardine and anchovy populations dissected into (a) high-frequency and (b) low-frequency components. The hindcast biomasses are based on calibration of the scale-deposition-rate series (Figure 2) to estimates of population biomasses in the central region of the California Current from 1935–1970. Separation of the series into its component frequencies indicates the distinct modes of variability over interdecadal and centennial time scales. The low-frequency component was obtained by low-pass filtering to remove all periods shorter than 100 years from the data. The high-frequency component is the difference between the unfiltered series and the low-frequency curve. Similar very-low frequency (millennial scale) change in both the sardine and anchovies is denoted (in b) by the long-term trendlines.

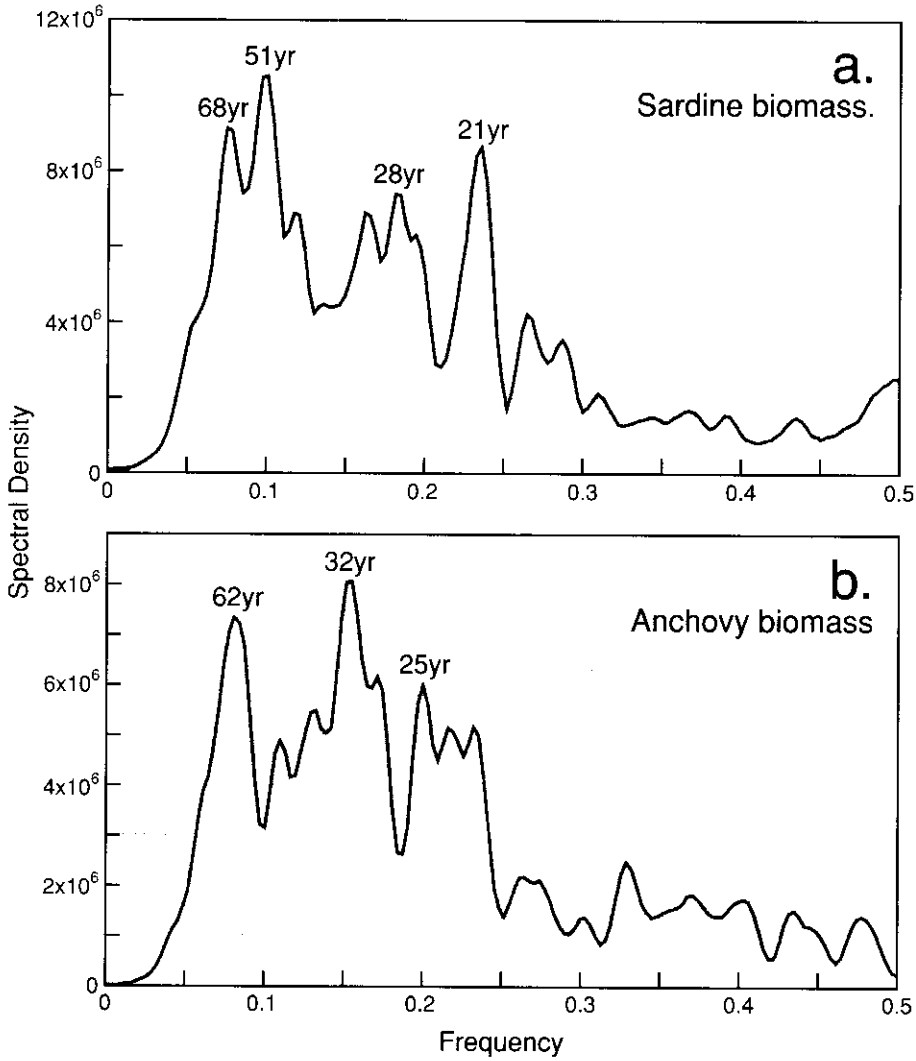

Figure 4. Variance spectra of the high-frequency component in the hindcast biomass series for the (a) sardine and (b) anchovy (biomass hindcasts in Fig. 3a). Comparison of this sardine spectrum to the corresponding spectrum from the original series (in Baumgartner et al., 1992) shows that the variance in the new series is now concentrated in a band with two peaks equivalent to periods around 50 and 70 years. This band roughly corresponds to the 76-year peak from the original sardine series (see Figure 11 in Baumgartner et al., 1992). The lower peak, centered around 30 years (in a) appears to correspond to the 57-year peak from the earlier sardine analysis. A third strong sardine peak is centered around 20 years. The anchovy spectrum (in b) shows two major peaks at roughly 30 and 60 years, corresponding to the 57- and 72-year peaks in the earlier analysis by Baumgartner et al. (1992). The 99-year peak in the earlier analysis is not present in the new anchovy spectrum.

Development of Background Environmental Information

We have developed histories of background environmental information from two principal sources during this project. These are the analyses of stable isotopes on selected foraminiferal species and the radiocarbon measurements on *Limacina helicina*. The radiocarbon measurements give an indication of variation in the rates of ventilation of radiocarbon which are associated with changes in ocean circulation and, in particular, with changes in large-scale upwelling (not shown here). We are now working on the interpretation of the ocean-reservoir ages obtained from the *Limacina*-wood differences to interpret the history of large-scale upwelling over the past 1500 years (e.g., Baumgartner and Southon, 1995).

The stable isotopes provide a means to extract a record of hydrographic conditions in which the foraminifera were living. Based on the sediment trap study in the Southern California Bight by Sautter and Thunnel (1991), and given the resources and time available, we concentrated our efforts on exploring the use of *O. universa* and *N. dutertrei*. These were extracted from the four thick slabs of KC 9110-1302 by wet seiving in the same process used for the fish scales, but were then separated and dried. Picking and preparing of the foraminifera for analyses was carried out by D. Field. Isotope analyses were done on the Finnigan/MAT-252 mass spectrometer at Scripps Institution of Oceanography with the cooperation of Christopher Charles and Ulysses Ninemann. Isotopic values are given in δ notation relative to the Chicago standard Pee Dee Belemnite (PDB) (Craig, 1957).

Extensive preliminary testing of shell size effects in our material demonstrated clear effects on both the $\delta^{18}O$ and $\delta^{13}C$ values in both *N. dutertrei* and *O. universa*. Unfortunately, the four thick slabs of KC 9110-1302 did not yield sufficient individuals of *O. universa* to meet our standard of a continuous sequence with at least two replicates from 5-year sampling intervals. However, comprehensive preliminary work comparing *N. dutertrei* and *O. universa* indicates that signals from *O. universa* and *N. dutertrei*—at this sampling frequency, in these sediments—may be redundant to a large degree. This comparison was made with a continuous sequence of 44 sample intervals (with two replicates wherever possible) with individuals of both species separated into two size fractions based on effects shown by Dunbar (1983). We used 5–6 individuals per replicate for *O. universa* and 6–7 individuals for *N. dutertrei*. Observations of similar isotopic signals given by *O. universa* and *N. dutertrei* in this comparison are given more weight by recent work of Ortiz et al. (1995) showing similar responses to varying hydrographic conditions, apparently regulated to an important degree by association with symbiotic photosynthesizing algae (Gastrich, 1988) in both species.

Based on the recommendation of Bouvier-Soumagnac and Duplessy (1985), we chose the larger size fraction (400–500 µm) of *N. dutertrei* for development of the final time series of isotopic analyses. Figure 5 demonstrates an overall negative correlation between the $\delta^{18}O$ and $\delta^{13}C$ values for *N. dutertrei* from the analyses completed so far. Our initial interpretation of this relationship is based on observations by Spero and Williams (1989) and Ortiz et al. (1995). We postulate that enrichment of $\delta^{13}C$ would result from upward migration of the individuals across the gradient of $\delta^{13}C$ of the seawater ΣCO_2 which increases towards the surface—and that this enrichment would overprint any change towards reducing the $\delta^{13}C$ gradient by upwelling. Enriched $\delta^{13}C$ values would be associated with relative depletion of ^{18}O (not affected by activity of the algal symbiont and in isotopic equilibrium with seawater), indicating generally warmer temperatures at shallower depths (as seen in Figure 5).

Figure 6 plots the variability of oxygen and carbon isotopes over a series of 104 samples of 5-year intervals (a total span of 520 years) beginning at approximately the year 700 AD. Given our interpretation above, the signals would be created mainly by vertical adjustment to light habitat, with limits varying between upwelling and more eutrophic, turbid conditions (low ambient light, population at shallow depths, enrichment of ^{13}C and relatively warmer *in-situ* temperatures) vs. stratified and more oligotrophic conditions with light penetration to greater depths (population deeper, with relative depletion of ^{13}C values with relatively cooler *in-situ* temperatures). This appears at first to be counter-intuitive, with upwelling resulting in relatively warmer temperatures; however, that is because calcification would occur relatively closer to the surface, which is warmer in a relative sense (even during upwelling) than the deeper water present during stratified conditions. Both the carbon and oxygen isotope series in Figure 6 show

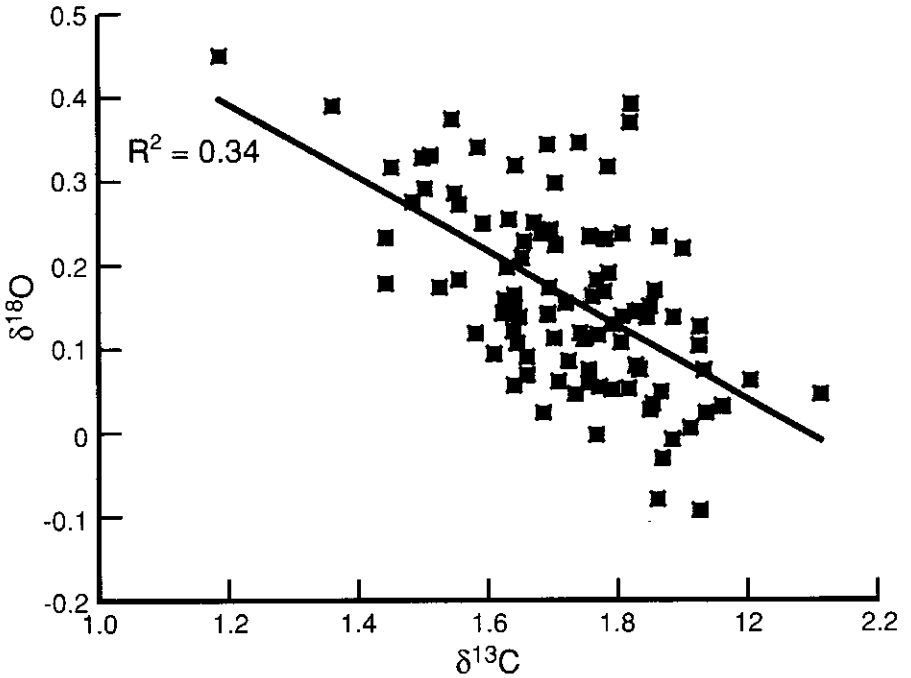

Figure 5. Scatter plot showing relationship between the $d^{18}O$ and $d^{13}C$ values for the 400–500 µm size fraction of *N. dutertrei*. These values are from the 5-year sample intervals from KC 9110-1302 and represent a span of 520 years, starting in approximately 700 AD and ending near 1220 AD.

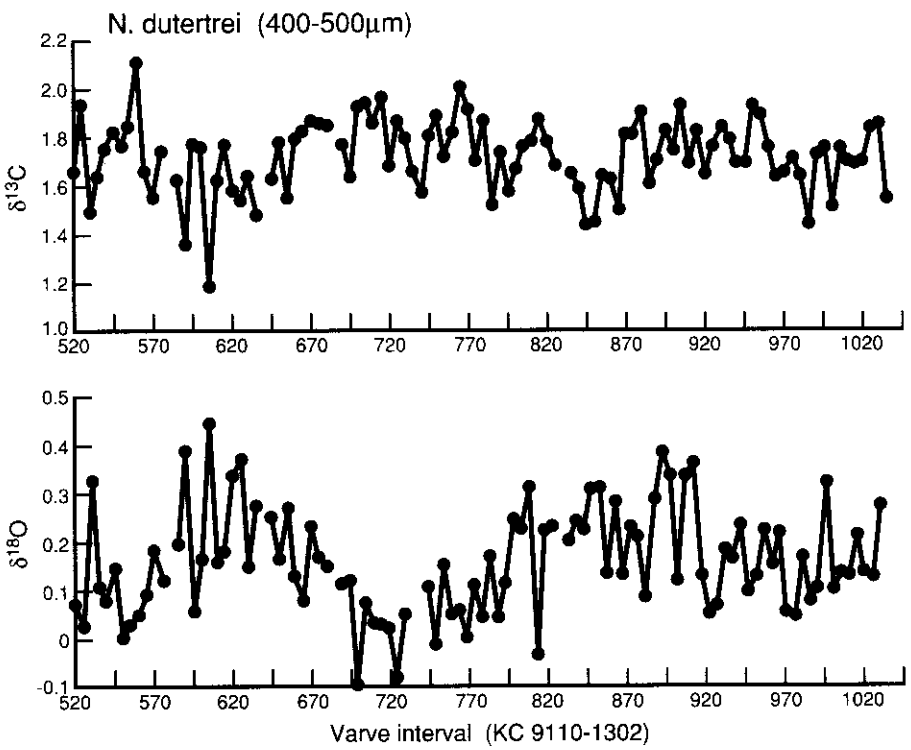

Figure 6. Time series of the $d^{13}C$ and $d^{18}O$ values for the 400–500 µm size fraction of *N. dutertrei*. These are averages of replicate samples from the 5-year sample intervals from KC 9110-1302. The time scale is given in terms of the varve-years from the chronology which is still under development for this core. This period of sampling corresponds to the period from 700 AD to 1220 AD.

strong multi-centennial-scale variability, which is consistent with that shown by the low-frequency plot for the sardines in Figure 3b. There is also a clear indication of interdecadal variability, particularly in the carbon isotope series with periods varying from 40 to 60 years.

Cooperating Organizations

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Wave-Driven Transport of Surface Oil Slicks

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R/CZ-118
1992–94

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The research has been organized into a sequence of tasks: prediction of surface wave drift in a steady progressive wave, parameterization of predictions for swell and directional sea conditions, and demonstration of the potential of surface wave drift in a coastal region.The first task is the theoretical prediction of the wave-averaged Eulerian surface drift under idealized conditions, namely for a steady progressive (and nonlinear) wave train. Theoretical surface wave kinematics have been obtained from high-order Fourier wave theory (Sobey, 1989). To all pragmatic purposes, this is an exact steady wave theory, provided only that appropriate attention is given to the truncation order and the number of computational nodes along the free surface (Sobey, 1988).

The Eulerian surface wave drift velocity at a particular position x is

$$W = \frac{1}{T}\int_0^T u\left(z = \eta,\ t;\ x\right)dt,$$

where u is the horizontal water particle velocity at the water surface $\eta(t;x)$ at time t, and T is the wave period. The integral was evaluated numerically from predictions of horizontal velocity at closely spaced points along the predicted water surface.

Results have been organized in a nondimensional framework,

$$\frac{\omega W}{g} = f\left(\frac{\omega^2 H}{g},\ \frac{\omega^2 h}{g}\right),$$

where ω is the wave frequency. The parameter range covers the complete spectrum of dimensionless wave height (ω^2H/g) and water depth (ω^2h/g) conditions that might be expected in practice. The present results are summarized in Figure 1. In dimensional terms, for example, the surface drift associated with a moderate 2-m high, 10-sec period wave in water 10 m deep is predicted to be 0.031 m/sec or 112 m/hr. For larger waves, the surface drift can be an order of magnitude larger. The dimensionless scaling for wave height in Figure 1 includes the Miche estimate for the limit wave,

$$H_{Miche} = 0.14L\tanh kh,$$

which was introduced to best resolve the expected variability in wave height under real sea conditions.

The second task is the parameterization of predictions for swell and directional sea conditions. For swell conditions, the sea state is long crested and quite narrow banded. There will be little variation about the mean height and period, which can be directly associated with the theoretical predictions of surface wave drift. The direction of the surface drift will be the local wave direction, as influenced principally by refraction in open coastal waters. For real sea states, a directional spectral description $E(\omega,\theta)$ has been adopted.

A directional spectral description of the local wave drift velocity follows directly from $E(\omega,\theta)$:

$$\bar{W} = \int_{-\pi}^{\pi}\int_0^{\infty} dW\left[E\left(\omega,\ \theta\right)d\omega d\theta\right].$$

In application, a discrete approximation provides estimates of both the magnitude and the direction of the local wave drift. The height to associate with the theoretical predictions of surface drift in each frequency, direction band has been assigned as $[8E(\omega,\theta)\Delta\omega\Delta\theta]^{1/2}$, the height of the equivalent steady wave.

The third task, the demonstration of the potential of surface wave drift in a coastal region, has a number of distinct components. These include a spectral wave model to predict the spatial and temporal variation in the nearshore wave field under incident sea forcing, an Eulerian advective and dispersive transport model for prediction of the spatial and temporal transport of a surface oil slick, and computer graphics animation of surface transport of an oil slick.

The spectral wave model follows well-established practice in wind–wave prediction (e.g., Sobey and Young, 1986) and is based on the radiative transfer equation,

$$\frac{\partial F}{\partial t} + \frac{\partial F}{\partial x_\alpha}\frac{\partial x_\alpha}{\partial t} + \frac{\partial F}{\partial k_\alpha}\frac{\partial k_\alpha}{\partial t} = Q\left(k_\alpha;\ x_\alpha,\ t\right).$$

The dependent variable is the variance spectral density $F(k_\alpha) = CC_gE(\omega,\theta)/\omega$, and $Q(k_\alpha)$ is a source term representing the net transfer of variance to, from, or within the spectrum at the wave number k_α due to all physical processes (apart from shoaling and refraction) that influence the component k_α. The variables C and C_g are the phase and group speeds, respectively, at wave number k. Grouped together with the simultaneous ray equations, the equivalent characteristic equations are

$$\frac{dF}{dt} = Q \qquad \frac{dk_\alpha}{dt} = -\frac{\delta\omega}{\delta h}\frac{\delta h}{\delta x_\alpha}$$

$$\frac{d\omega}{dt} = 0 \qquad \frac{dx_\alpha}{dt} = C_{g\alpha}.$$

These are the equations on which the discrete spectral model is based.

Solutions are sought at discrete nodal points ($i\Delta x, j\Delta y$) throughout the solution field at times $t_0 + n\Delta t$, subject to initial conditions at time t_0 and boundary conditions for all time. For discrete computational purposes, the directional energy–density spectra at each node and time are

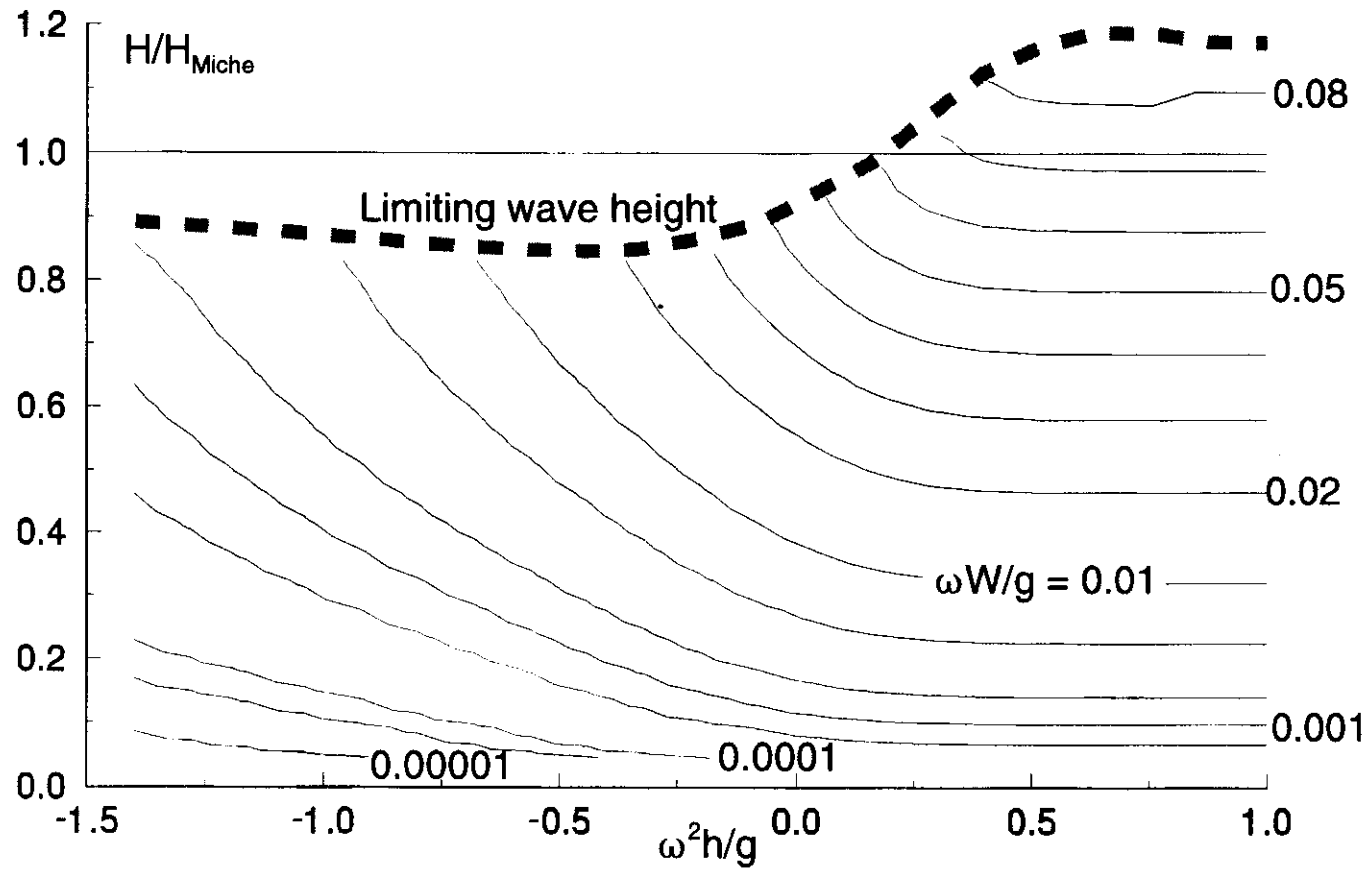

Figure 1. Eulerian surface wave drift.

represented as a summation of separate frequency and direction bands:

$$\iint E\left(\omega,\ \theta;\ x_\alpha,\ t\right)d\omega d\theta = \sum_k \sum_\ell E\left(\omega_k,\ \theta_\ell\right)\Delta\omega_k\Delta\theta.$$

Ten frequency and 16 direction bands have been adopted to adequately resolve the directional spectrum at all stages of growth and evolution.

A fractional-step algorithm has been adopted, for which purposes the radiative-transfer equation may be written as

$$\frac{\partial}{\partial t}\left(CC_gE\right) = \left(A_1 + A_2\right)\left(CC_gE\right).$$

The two operators A_1 (propagation, refraction, and shoaling) and A_2 (forcing) are given separate and consecutive consideration over each time step. The propagation step advances the solution from E^n to $E^{n+1/2}$ through solution of the partial differential equation

$$\frac{\partial}{\partial t}\left(CC_gE\right) = 2A_1\left(CC_gE\right).$$

The forcing step then advances the solution from $E^{n+1/2}$ to E^{n+1} through solution of the partial differential equation

$$\frac{\partial}{\partial t}\left(CC_gE\right) = 2A_2\left(CC_gE\right) = 2CC_g\left(a + bE\right),$$

where advantage has been taken of the common representation of all source terms in the form $a + bE$. Forcing traditionally (Sobey and Young, 1986) includes atmospheric transfer, non-linear interactions, and dissipation. In this case also it includes filtering of the waves by surface oil at very high frequencies (Gottifredi and Jameson, 1968). A numerical solution for the forcing step is unnecessary, because a local analytical solution is available:

$$E^{n+1} = E^{n+1/2}\exp\left(b\Delta t\right) + a\Delta t\left[\frac{\exp\left(b\Delta t\right) - 1}{b\Delta t}\right].$$

A numerical method of characteristics coupled with third-order interpolation has been adopted for the propagation step.

An initial step in this solution is computing the wave orthogonals for each discrete frequency in each direction band at each computational node. In the absence of current refraction, the propagation medium is time independent, and the ray computations need to be done only once for any solution field. The spatial and temporal resolution adopted is considerably finer than Δx, Δy, and Δt, permitting resolution of shoaling, refraction,

and ray curvature in transitional and shallow water.

For demonstration purposes, the code has been applied to a schematic nearshore bathymetry that has a ridge feature normal to the shore, as shown in Figure 2. The incident sea is a Pierson–Moskowitz spectrum with cosine–to–the–fourth directional spread and variance of 1 m^2. The incident wave direction is 60° to the x axis. The arrows in Figure 2 are surface transport vectors.

A parallel coding effort has been directed toward the Eulerian transport model for the surface oil. This is based on the advection–dispersion–reaction equation

$$\frac{\partial C}{\partial t} + W_\alpha \frac{\partial C}{\partial x_\alpha} = \frac{\partial}{\partial x_\alpha}\left(E_\alpha \frac{\partial C}{\partial x_\alpha}\right) + \Sigma,$$

where C is the local concentration of the surface oil (=$\Delta\rho d$, where $\Delta\rho$ is the local mass density deficit of oil w.r.t. water, and d is the local thickness of the oil layer), E is the local dispersion coefficient of the surface oil, and Σ represents the net source term contributed by other influential processes. The dominant term will be advection by wind, ambient current, and waves. Dispersion processes include buoyant spreading, vertical shear flow dispersion, and turbulent diffusion. Included among the source terms are a range of weathering processes (e.g., evaporation, emulsification, biodegradation). Except for wave drift, all these processes are well-recognized influences in the transport of oil slicks. They would be included in a complete model but are excluded in the present analysis to permit focusing attention on the wave transport.

Numerical difficulties in the solution of a transport equation of this form can be severe. Extensive numerical dispersion and solution oscillations may result if adequate attention is not given to the numerical algorithm. The fractional-step algorithm of Sobey (1983), as extended to two horizontal spatial dimensions by Bode and Sobey (1984), has been adopted. This algorithm provides a near-exact solution and avoids the common numerical difficulties.

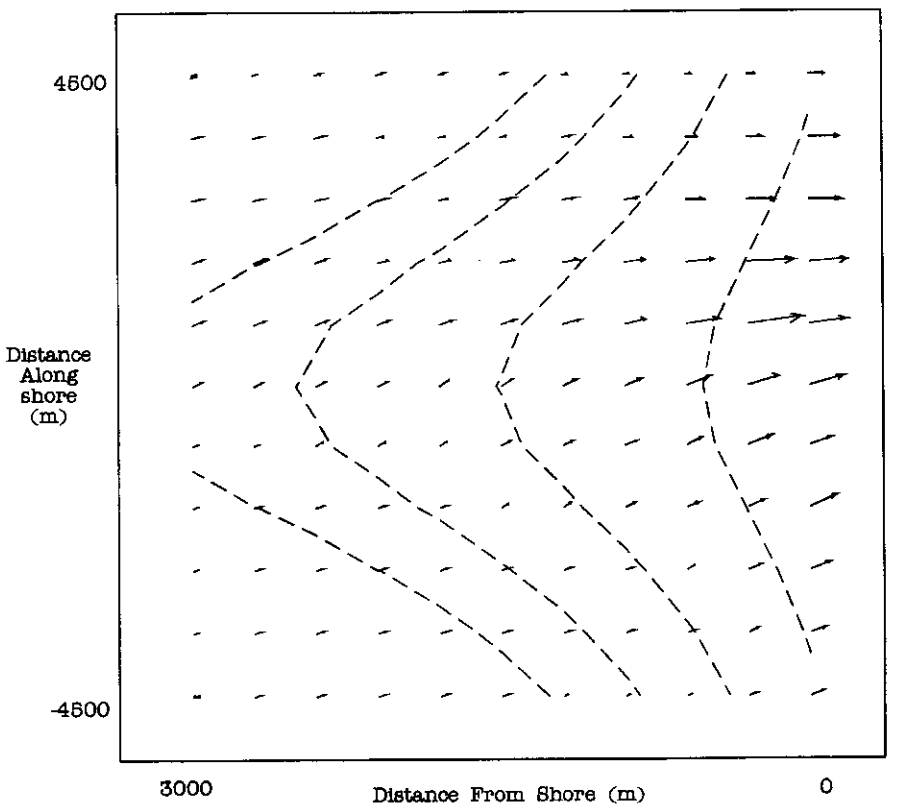

Figure 2. Bathymetry contours and surface velocity vectors for a peak offshore wave direction of 60°.

In the fractional–step algorithm, the advection step advances the solution from C^n to $C^{n+1/3}$ by solution of the partial differential equation

$$\frac{\partial C}{\partial t} = 3A_1 C.$$

A moving coordinate algorithm with fourth-order Runge–Kutta was adopted. The dispersion step

$$\frac{\partial C}{\partial t} = 3A_2 C$$

was solved by a finite difference method on the irregular grid defined by the advection step. The reaction step advances the solution from $C^{n+2/3}$ to C^{n+1} from solution of the partial differential equation

$$\frac{\partial C}{\partial t} = 3A_3 C = 3\left(K_0 + K_1 C\right),$$

which has a local analytical solution for zeroth and first order reaction models.

The final phase of the third task was computer graphics animation of the surface transport of an oil slick. Scenarios were based on a schematic nearshore bathymetry (Figure 2) and a range of expected environmental conditions that will include wind and current forcing as well as waves. Figure 3 is a typical example. It shows four consecutive stages in the wave-driven transport of an oil slick in the wave field depicted in Figure 2.

The oil slick is represented by a sequence of distinct nodes. Convergence of the nodes indicates an increase in the concentration of the oil slick (thickening of the oil layer), and divergence of the nodes indicates a thinning of the oil layer. For the incident Pierson-Moskowitz spectrum with a variance of 1 m^2 shown in Figure 2, an oil slick 8 km from shore will be beached in approximately 42 hours. With a longshore current of 0.1 m/sec and the same incident sea, the same spill would be beached in approximately the same time; this is the situation depicted in Figure 3. Without an onshore wind, the

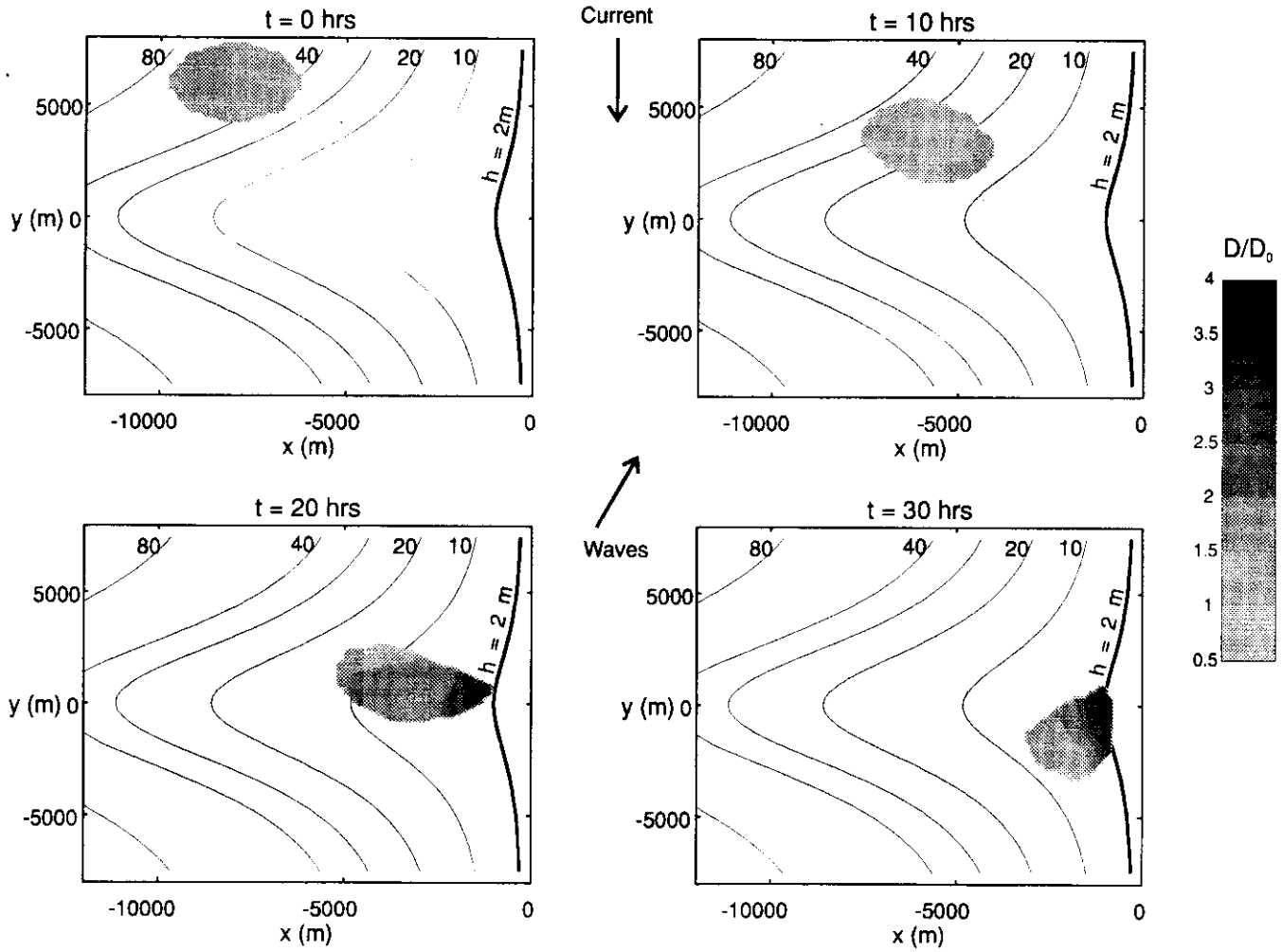

Figure 3. Wind-driven transport of an oil slick in wave field shown in Figure 2.

beaching of surface oil by natural refraction processes in the nearshore is inevitable.

Cooperating Organizations

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Aquaculture

Hormonal Regulation of Crustacean Growth and Reproduction

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Lobster Neuropeptide Hormones

Synthesis or secretion or both of ecdysone by Y-organs in lobsters is inhibited by the neuropeptide molt-inhibiting hormone (MIH; Chang, 1989). MIH is synthesized by the eyestalk X-organ neurons and stored in the sinus gland, a neurohemal organ. The complete amino acid sequence of lobster (*Homarus americanus*) MIH was reported by our laboratory (Chang et al., 1990). A closely related neuropeptide from the sinus gland of the lobster eyestalk is crustacean hyperglycemic hormone (CHH). In collaboration with David Schooley (University of Nevada, Reno), we have determined the amino acid sequence of this hormone in lobster. We found a 96% identity between CHH and MIH.

These lobster peptides are members of a novel family of crustacean neuropeptides that also includes a vitellogenesis-inhibiting hormone (VIH; Soyez et al., 1991). Several researchers have recently published the results of studies on hormones from this family (Huberman and Aguilar, 1988; Kegel et al., 1989; Chang et al., 1990; Soyez et al., 1991; Tensen et al., 1991a, 1991b; Webster, 1991). We have established working ties with colleagues who are studying this family in other species. We have exchanged hormones and antisera made against these peptides.

We constructed a cDNA library made from lobster eyestalks. Sinus glands and surrounding neural tissue were dissected from the eyestalks and frozen in liquid nitrogen. RNA was isolated from the tissue homogenates (Chomczynski and Sacchi, 1987), and the mRNA was separated from total RNA.

Complementary DNA was made from the purified mRNA and ligated to phage lambda arms using a ZAP-cDNA kit (Stratagene). These recombinant phage were then added to bacterial lawns of *Escherichia coli* to produce plaques. The plaques were lifted with nitrocellulose filters and then screened with a ^{32}P-labeled oligonucleotide probe. The probe was constructed by using a polymerase chain reaction (Saiki et al., 1988) and primers complementary to amino acid residues 11 through 67 of MIH. X-ray film was exposed by the radioactivity on the filters and used to identify colonies of bacteria containing lobster DNA that codes for a member of the MIH/CHH neuropeptide family.

We isolated several colonies and are determining the nucleotide sequence of these MIH/CHH/VIH genes. Information from these experiments is necessary for understanding and manipulating the regulation of these important genes.

Ecdysteroids and Molt Inhibition

It has been observed for many years that injections of 20-hydroxyecdysone can shorten the intermolt period in lobsters when the injections are given before premolt (Rao et al., 1973). We found, however, that a single injection of exogenous 20-hydroxyecdysone during premolt results in a subsequent delay in molting (Cheng and Chang, 1991). We did similar experiments in which we used multiple injections.

Full-sibling juvenile *H. americanus*, (11.7 g wet weight) were used for these experiments. Stage D_3 lobsters were identified on the basis of degree of cuticle digestion along the dorsal midline of the carapace and on the dorsal surface of the merus of the chelipeds (Cheng and Chang, 1991). Lobsters received daily injections of 20-hydroxyecdysone until the animals molted. Control animals received saline only. The time of molting was recorded by using time-lapse video with a time stamp. The lobsters injected with hormone molted 120 ± 45.1 hours (mean ± standard deviation) after the injections. The lobsters injected with saline molted 56.4 ± 28.1 hours after the injections. The difference between the two groups is statistically significant. No significant differences between control and experimental groups were detected when injections were given before or after stage D_3 (Cheng and Chang, 1991). Thus, both an initial increase in the concentration of circulating hormone and a coordinated decrease are necessary for successful molting.

Methyl Farnesoate As a Molt Stimulator

On the basis of the similarities between crustaceans and insects in endocrine regulation of molting, researchers hypothesized that an analogue to the insect juvenile hormone might be present in crustaceans. Recent work indicates that a sesquiterpenoid other than juvenile hormone may be the modulator of crustacean development. The related compound, methyl farnesoate, is a product of the mandibular organ and was isolated from the hemolymph of a number of decapod crustaceans (Laufer et al., 1987; Borst et al., 1987).

We suspected that, as in many endocrine systems, both a stimulator and an inhibitor would be involved in secretion of ecdysteroid by the Y-organ. In collaboration with Wallis Clark, we observed that secretions from the mandibular organ shortened the molt cycle. This shortening was not due to secretion of ecdysteroids by the organ (Yudin et al., 1980).

We tested the effects of methyl farnesoate on production of ecdysteroid both *in vitro* and *in vivo*. Molting glands from the commercially important Dungeness crab,

Cancer magister, were dispersed with collagenase, and the cells were cultured in the presence or absence of intact crab mandibular organs. The culture medium was assayed for ecdysteroids (Chang and O'Connor, 1979). Dispersed cells cocultured with a mandibular organ secreted significantly higher amounts of ecdysteroids into the culture medium. We observed that this stimulation is specific for the *cis,trans* isomer of methyl farnesoate and that the Y-organ responds in a dose-dependent manner (Tamone and Chang, 1993).

For *in vivo* experiments, methyl farnesoate was added to culture water containing first-stage larval lobsters. At various times in development, extracts of these larvae were assayed to determine the level of ecdysteroids. After 48 hours, larvae incubated with exogenous methyl farnesoate had significantly higher levels of ecdysteroids compared with control larvae cultured with hormone vehicle only (1.68 vs. 0.75 ng ecdysteroids per larva; $P < .005$; Chang et al., 1993).

In collaboration with David Borst (University of Illinois, Normal), we have isolated a peptide hormone from the lobster sinus gland that inhibits the production of methyl farnesoate by the mandibular organ. This hormone acts as an additional molt-inhibiting factor, because it prevents the synthesis and release of the molt stimulator methyl farnesoate (Borst et al., 1991; Tsukimura and Borst, 1992). We are determining the amino acid sequence of this potentially important molt regulator.

Methyl Farnesoate–Binding Proteins

Further evidence for an endocrine role of methyl farnesoate in crustaceans was the demonstration of a hemolymph binding protein for methyl farnesoate in both shrimp and lobsters (Prestwich et al., 1990). Using an analogue of methyl farnesoate (synthesized by our colleagues Ujváry and Prestwich, 1990), we measured the amount of the analogue bound by hemolymph obtained from lobsters at various times during the molt cycle. We observed significant changes in the amount of binding during the molt cycle. Binding was low in postmolt, increased during intermolt and early premolt, and slightly declined at late premolt. This level of binding closely follows the concentration of ecdysteroids in the hemolymph during the molt cycle (Chang and Bruce, 1980). The relationship between the binding of methyl farnesoate and the concentration of ecdysteroids remains to be determined.

Comparative studies were started on the protein in the crab (*C. magister*) that binds methyl farnesoate. It appears that the native protein is large (100–300 kD) and is composed of two different subunits. We have purified a large quantity of the protein and will determine the N-terminal amino acid sequence. These sequence data will permit comparisons with the known proteins that bind juvenile hormone.

Ecdysteroid Receptors

In collaboration with A.J. El Haj (University of Birmingham, England), we began a study on the role of ecdysteroids in muscle development in lobsters. Part of this study involved examining tissues at various molt stages for the presence of receptors for ecdysteroids. We observed that ecdysteroid receptors could be detected in premolt leg muscle but not at other molt stages (Figure 1). In addition, strong immunoactivity was detected in the eyestalk. This last observation implies that feedback communication may occur between the circulating ecdysteroids and the X-organ/sinus gland that produces MIH.

Cooperating Organizations

Stanford University
State Lobster Hatchery and Research Station, Vineyard Haven, Massachusetts
State University of New York, Stony Brook
University of Birmingham, England
University of Illinois, Normal
University of Nevada, Reno

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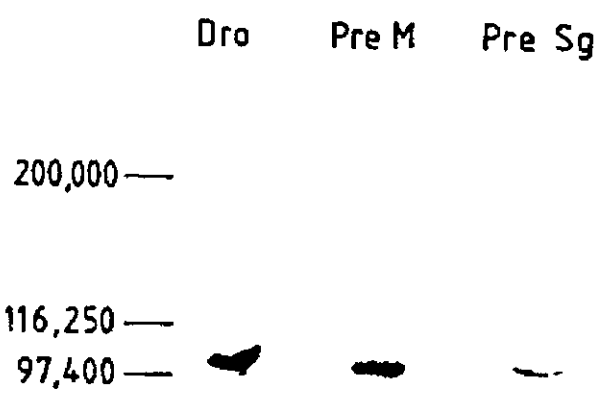

Figure 1. Western blot of extracts from *Drosophila melanogaster* embryos (Dro) and extensor muscle (Pre M) and X-organ/sinus glands (Pre Sg) from premolt lobsters. Samples were electrophoresed in a 12.5% acrylamide gel. Proteins were transferred to Hybond C Super membranes (Amersham) using a Transblot system (BioRad). Immunodetection was carried out using an enlaced chemiluminescence detection system (Amersham) and a monoclonal antiserum raised against a *D. melanogaster* EcR-TRpE fusion protein (Koelle et al., 1991). Broad molecular weight markers span 97,000 to 200,000 daltons.

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Development of Technologies for Long-Term Storage and Genetic Manipulation of Penaeoidean Eggs

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R/A-81
1990–93

Wallis H. Clark, Jr. and John H. Crowe

Techniques commonly used by agriindustry and health sciences to manipulate and store gametes have not been adapted for the culture of penaeids (prawns). The primary reason for this is a lack of information on penaeid reproductive biology in general. Penaeid gametes are markedly atypical compared with the better understood gametes of other animal groups, and techniques developed for the latter have not been directly transferrable (Clark and Griffin, 1993). Research in our laboratory on the reproductive biology of the penaeoidean shrimp *Sicyonia ingentis* has provided fundamental information on the mechanisms of sperm capacitation and activation and the interaction between sperm and egg (reviewed by Clark et al., 1984; Clark and Griffin, 1988; Pillai et al., 1990; Clark and Pillai, 1991). Our long-term goals are to continue using *S. ingentis* as a model for understanding fertilization in penaeoideans and reproductive phenomena in general. We hope that eventually our findings will make domestication of penaeoideans possible, so the aquaculture industry will no longer have to rely on seed stock or mated females from the wild. We have broadened our fundamental understanding during the past 3 years by examining the physiology of egg activation.

In the first year, we developed some techniques that facilitated our proposed work, and we obtained some exciting and unexpected results: (1) We discovered cytoskeletal changes associated with egg activation, fertilization, and early embryonic development and began examining the same changes in nonfertilized and polyspermic eggs. (2) We showed that cytoskeletal changes observed during egg activation do not occur in eggs spawned into artificial seawater that lacks magnesium. (3) We found that eggs spawned into this magnesium-free seawater can be rescued and we determined how long eggs can be maintained in this liquid and still remain viable. (4) We developed protocols for fixing, labeling, and clearing eggs for imaging on the laser scanning confocal microscope, and we developed techniques to quantitatively visualize intracellular ionic changes (e.g., pH, levels of calcium) within live eggs. (5) We successfully microinjected a fluorescent probe for calcium (Fluo-3) into live eggs, activated the eggs, and imaged two temporally separated releases of calcium.

In the second year, our accomplishments included the following. (1) We confirmed that magnesium acts externally on *S. ingentis* eggs to elicit activation. (2) We found that an increase in intracellular pH occurs as a result of activation when eggs have contact with extracellular magnesium. (3) We described and analyzed early activational changes (those that occur before eggs round up). (4) We determined that the times microtubular and nuclear changes associated with egg activation occur are not altered by the presence or absence of a fertilizing sperm. (5) We showed that eggs maintained unactivated in magnesium-free artificial seawater for extended periods are polyspermic.

During the last year of the project, we have successfully used artificial means to separate early cortical changes in eggs (contraction and rounding up) from meiotic changes; shown that the lag time between exposure to magnesium and egg activation is a function of the concentration of magnesium in the seawater, but that there is no threshold concentration of magnesium necessary for egg activation; and obtained further evidence that external magnesium interacts with a receptor on the egg surface to initiate egg activation.

From previous studies of gross morphology (Clark et al., 1984; Pillai and Clark, 1987; Clark et al., 1990) and from studies of the nucleus and cytoskeleton done in the first year of the project (see Lindsay et al., 1992; Hertzler and Clark, 1992), we have developed an understanding of changes associated with egg activation and the timing of those changes in *S. ingentis* (Figure 1). On exposure to or contact with seawater containing magnesium, eggs initiate a cascade of activational events that begins with a wavelike internal release of free calcium approximately 5 sec after contact with magnesium. After the release of calcium, a series of cortical changes occurs. Within 5 min or less, jelly precursor exits from extracellular cortical crypts. As the crypts disappear, the egg increases in size and then contracts and assumes a spherical shape (i.e., rounds up). About 15–20 min after contact with magnesium, resumption of meiotic maturation is evidenced by a 90° rotation of the meiotic spindle and metaphase I chromosomes. Extrusion of the first polar body occurs at 35–40 min, a hatching envelope is elevated at 40–45 min, and the second polar body is released (signaling the completion of meiotic maturation) at 45–50 min. The absolute timings of these activational events are temperature dependent. The times given here are representative for a temperature of 20–22°C.

Continued experiments on the role of magnesium in the activation of *S. ingentis* eggs have supported and extended earlier results showing that in the absence of extracellular magnesium, activation does not occur and that the mechanism of this activation is via an extracellular interaction between magnesium and the egg plasma membrane (Lindsay

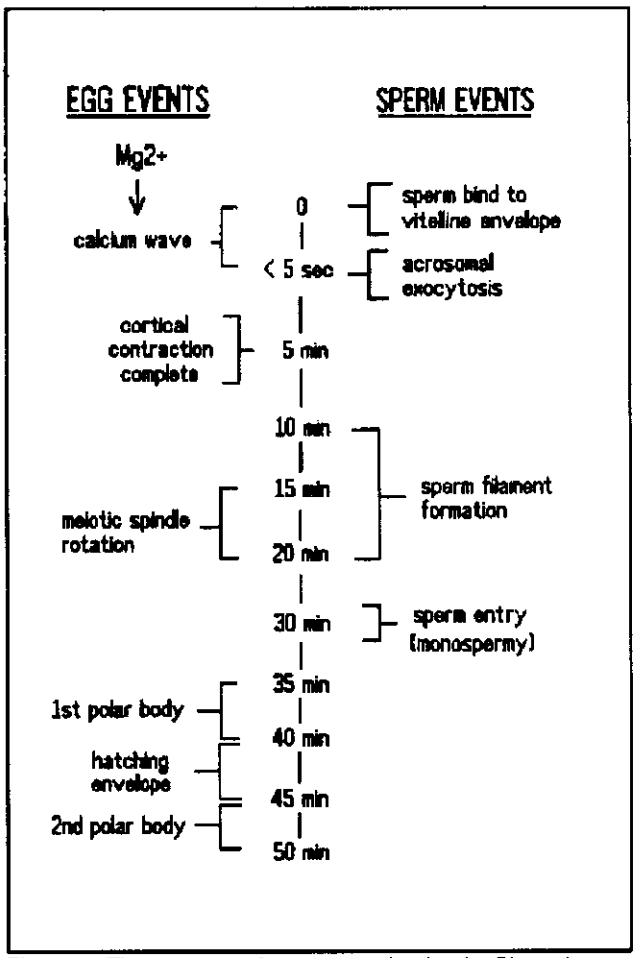

Figure 1. Time course of gamete activation in *Sicyonia ingentis*. From Lindsay et al., 1992.

et al., 1992). We have been able to use manganese as a substitute for magnesium in the seawater to further demonstrate this supposition. Manganese initiates an egg activation that is indistinguishable from one induced by magnesium. Manganese also has the unique property of quenching the emitted fluorescence of the Fluo-3–calcium complex. Therefore, the prediction would be that the calcium wave would not be observed if manganese were entering the egg at activation. A normal calcium wave is observed, indicating that manganese is acting extracellularly.

We determined that the time required for an egg to complete the cascade of activational events is related to the extracellular concentration of magnesium. Earlier reports on the role of magnesium in activation of crustacean eggs concluded that a minimum threshold concentration of magnesium in the millimolar range (15 mM) was necessary for activation (Goudeau and Goudeau, 1986). Although *S. ingentis* eggs will not activate if *no* magnesium is present, they will activate, albeit in a delayed fashion, in external concentrations of magnesium less than or equal to 10 mM. Eggs exposed to concentrations of 50 mM (near the normal concentration in seawater) activate and proceed to form a hatching envelope at 40–45 min (see Figure 1). In experiments with reduced concentrations of magnesium, a hatching envelope formed at 42.7–47.7 min in concentrations of 10–50 mM. Although the time required increased as the concentration of magnesium decreased, differences with 10–50 mM were not significant. Envelope formation was, however, greatly delayed in concentrations of 5 and 1 mM, occurring at 58.6 and 102.5 min, respectively. Results also suggest that normal fertilization and development do not require normal seawater concentrations of magnesium; eggs activated and fertilized in concentrations of magnesium as low as 5 mM proceed through a normal first cleavage. Also, as with formation of a hatching envelope, the time at which cleavage occurred was affected by the extracellular concentration of magnesium. Cleavage required 98.6 min in seawater containing 5.0 mM and only 81.7 min when the concentration was 50.0 mM. Eggs incubated in a concentration of 1 mM, undergo activation through formation of a hatching envelope and extrusion of a polar body, but do not go on to first cleavage, indicating that development may not occur at levels of magnesium of 1 mM or less. We do not yet know if external magnesium is required only to trigger the initial events of the cascade (e.g., the intracellular calcium wave) or if it must be present throughout activation. The increase in time between activational events that occurs as the concentration of magnesium in seawater is reduced provides an ideal system for dissecting and examining the controls and relationships between events in egg activation.

In addition to showing that manganese can substitute for magnesium and induce egg activation, we have found that other divalent cations can elicit partial or delayed activational events, thus providing more avenues for separating activational events. Either of two cations, strontium and nickel, initiated a delayed release of calcium in a percentage of eggs. A number of cations (strontium, nickel, barium) elicited egg contraction and rounding up, and strontium and barium even elicited partial formation of a hatching envelope. In addition, all divalent cations mentioned here, except calcium and zinc, triggered the resumption of meiotic maturation. However, meiotic maturation was completed and was normal only with manganese.

Our experiments showing that magnesium acts externally to induce the calcium wave and other activational events also suggest that eggs may contain a receptor for magnesium and that the interaction

of magnesium and this receptor activates eggs. Most likely, a threshold number of receptors must be bound by magnesium before activation occurs. Therefore, in reduced concentrations of magnesium, more time would be required to activate the threshold number of egg-borne receptors for magnesium. This possibility is exciting, has precedence in other cell types (Brown, 1991), and may be applicable to other marine invertebrates that are exposed to extracellular multivalent ions before a cellular activation or change.

We compared activation and the timing of activational events in fertilized and nonfertilized eggs. Although early indications suggested that activational events might be slower in nonfertilized eggs than in fertilized eggs, more rigorous examination has shown otherwise. Using the laser confocal scanning microscope and the fluorescent probe Fluo-3, we found no significant difference in the timing of the initial release of intracellular calcium or in the characteristics of the wavelike release propagated throughout the cell. Image 1 analyses of video recorded activations revealed no differences in the timing or characteristics of other events such as cell contraction and rounding up, elevation of a hatching envelope, and extrusion of a polar body. Therefore, we conclude that the cascade of activational events in *S. ingentis* eggs is initiated by contact with magnesium ions in seawater and uses the same mechanisms regardless of whether a fertilizing sperm has contacted the egg. Although eggs require external magnesium, sperm do not and will activate on eggs in magnesium-free conditions. We have confirmed that unactivated eggs (in magnesium-free seawater) are incapable of regulating entry of sperm and such eggs become polyspermic (Lindsay et al., 1992).

We have documented and quantified changes in shape and size that occur in *S. ingentis* eggs before the completion of cortical contraction (i.e., rounding up; see Figure 1). These data were obtained by using video microscopy and our Image 1 image analysis hardware and software. Quantitation of changes in size was based on measurements of areas on images obtained at 20X magnification to ensure that the widest diameters of eggs were captured. The program used accommodates variable shapes which allows easy and accurate comparison of areas through changes in an egg's shape. Before release and activation, eggs may be ovoid to irregular, cover a two-dimensional surface area of 0.047 mm^2, and have extracellular cortical crypts 40–50 μm deep that contain jelly precursor (Clark et al., 1990). This measurement (0.047 mm^2) reflects the overall area, including that area encompassed by crypts and crypt material. Within 30 sec of contact with seawater containing magnesium, the depth of the extracellular crypts begins to decrease, and extrusion of jelly precursor occurs. As the depth decreases, overall egg size (area) increases and may reach 0.052–0.055 mm^2.Then, approximately 1–2 min after contact with magnesium, the eggs reverse this trend and contract. By 3 min after spawning, the area has decreased to 0.040–0.043 mm^2, and after this decrease in size, eggs change shape and round up. We suspect that increases in cell size are the result of membrane added to the cell periphery during the period of crypt disappearance. Contraction and rounding up could then be attributed to changes in the cortical cytoskeletal and the internalization of the plasma membrane, as hypothesized for the related species *Penaeus aztecus* (Clark et al., 1980).

Sicyonia ingentis eggs undergo an intracellular alkalinization as a result of exposure to extracellular magnesium at activation. This assertion is supported by experiments that showed activation is accelerated in seawater with elevated pH and retarded in seawater with low pH. If eggs are spawned into and incubated in seawater with depressed pH (pH 7.0 or below), activation is retarded and appears to be incomplete. Such eggs contract, round up, and resume meiotic maturation, as shown by extrusion of the first polar body. They also form a hatching envelope; however, it does not elevate from the surface of the egg. This indicates that formation is incomplete. Release of the second polar body has not been observed in seawater at pH 7.0. However, it is not yet clear if this represents incomplete meiotic divisions. In contrast to the effects of depressed external pH, an increase in the pH of seawater accelerates and exaggerates the activation process. At pH 9.0, formation of a hatching envelope occurs earlier, and the envelope elevates farther than in seawater of pH 8.0. Measurements obtained with the aid of the Image 1 package showed the following: (1) the size of the egg after formation of a hatching envelope was not altered by external pH (egg area ranged from 0.040 to 0.353 mm^2), and (2) the elevation of the hatching envelope from the egg surface doubled, from 26.2 μm to 47.2 μm, when eggs were spawned into and incubated in seawater of pH 9.0 compared with seawater of pH 8.0. Although certain activational events are accelerated and enhanced when external pH is increased, we have not observed embryonic development in these eggs. This could be due to as yet undetermined adverse effects of pH on the eggs or, more likely, sperm may be prevented from completing the acrosome reaction and thus fertilizing an egg in seawater of pH 9.0. We have previously shown that sperm undergo an internal acidification during the acrosome reaction and that the second phase of the acrosome reaction can be inhibited by high external pH (Griffin et al., 1987; Lindsay and Clark, 1992).

Our work to date strongly suggests that although the events of egg activation in *S. ingentis* may be a cascade, events within the same apparent cascade may be under different controls. That is, certain events may be triggered by the increase in intracellular free calcium, and others may be under the control of an internal shift in pH or an as yet undisclosed mechanism. Interesting and exciting results in

experiments in which both external pH and concentrations of magnesium have been manipulated support the aforementioned idea. Previous work had shown that eggs spawned into and incubated in magnesium-free seawater did not activate. We asked if the maintenance of eggs in the unactivated state in magnesium-free seawater was pH dependent. Our assay in initial experiments was egg contraction and rounding up. At pH 8.0 and above, fewer than 15% of the eggs rounded up. As external pH decreased, the percentage of eggs that rounded up increased, and at pH 6.5, 89% of the incubated eggs rounded up. These results suggest that if we are observing a physiologically relevant response, *S. ingentis* eggs may undergo multiple shifts in intracellular pH during activation.

Cooperating Organizations

California Department of Fish and Game, Santa Barbara
Centre Oceanologique du Pacifique, Tahiti, French Polynesia
Louisiana State University, Baton Rouge
Louisiana State University Medical School, New Orleans
McCormick & Associates, Ojai, California
National Institutes of Health, Bethesda, Maryland
National Taiwan University, Republic of China
Tungkang Marine Laboratory, Republic of China
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Sonoma-Marin Marine Advisory Committee Meetings. Held in various locations throughout Sonoma/Marin Counties every other month.

Hormone Stimulation of Reproductive Maturation in Female Sturgeon

University of California, Davis
R/A-85
1991–94

Gary P. Moberg

Because of collaborative efforts between researchers at the University of California and aquaculturists, a new industry that produces highly prized white sturgeon has been established in California. However, further development of this industry depends on successful and reliable domestic breeding of the captive broodstocks established by farmers. Since 1987 aquaculturists have used the semen of cultured males for artificial reproduction, and recently some cultured females have reached sexual maturity at 7–10 years of age and have been successfully spawned, indicating that captive breeding is feasible. However, monitoring of ovarian maturation in a large number of farmed broodstock indicates that the major problems in the management of sturgeon broodstock are the late age of puberty, variable recruitment into vitellogenesis among fish of the same age, and the influence of environmental conditions on final ovarian maturation.

Economically efficient breeding of sturgeon will depend on the early onset of puberty, synchronous onset of vitellogenesis in fish of the same age group or size, and normal final ovarian maturation. Despite the late maturity and long reproductive cycle in wild acipenserid stocks, our collaborative work with industry suggests that a 30% maturation and spawning rate in cultured white sturgeon females 6–7 years old may be achievable and fully adequate to start commercial production of fingerlings. To accomplish this result, we have focused on the role of the hypothalamic-pituitary-gonadal axis in controlling reproduction and on the influence of the environment in regulating the reproductive cycle of cultured sturgeon.

In 1988 we began to study endocrine regulation of sturgeon reproduction (Sea Grant R/A-73). Our goals were to develop methods for manipulating the reproduction of cultured female sturgeon and to obtain basic information about the endocrine control of this primitive ray-finned fish. The sturgeon (Chondrostei) is perhaps the closest among living fish to the common ancestor of modern teleosts and tetrapods. The reproductive physiology and development of sturgeon have many features similar to those of amphibians and mammals, such as the structure of the gonadotropin-releasing hormone (GnRH), nucleotide and derived amino acid sequences in the vitellogenin gene, and embryonic cleavage (Detlaf et al., 1981; Bidwell, 1989; Sherwood et al., 1991). In our studies, we have identified two sturgeon pituitary gonadotropins that control reproduction (Moberg et al., 1991b). Yet, many aspects of how secretion of the gonadotropins is regulated remain obscure and need to be understood before we will be able to control sturgeon reproduction.

The overall objective of the project is to establish full reproduction of white sturgeon raised in commercial aquaculture by bringing females into reproductive maturity, synchronizing vitellogenesis, and inducing final maturation by controlling the secretion of pituitary gonadotropins. Using our previously developed techniques, we are defining the basic control of gonadotropin secretion in order to create endocrine treatments that can be used to induce full reproductive maturation of female sturgeon.

Gonadal Development

We have made significant progress in understanding the reproduction and endocrinology of cultured female sturgeon, which is an essential step for establishing full reproduction in these animals. With our annual monitoring of the plasma concentrations of hormones and the ovarian development of more than 500 females maintained at several cooperating aquaculture facilities, we have been able to follow the reproductive development of maturing females and are now able to describe gonadal development in these fish. By 2–3 years of age, white sturgeon become sexually differentiated. The gonial proliferation phase is completed by 3–4 years of age. Primary oocytes reach 200–300 μm in diameter and are surrounded by a basal lamina and differentiated thecal layer. They remain in this refractory stage for a variable time (1–5 years before the onset of the vitellogenic cycle). Three successive morphological changes characterize the onset of the cycle: (1) proliferation of granulosa cells and differentiation of the inner follicular layer; (2) synthesis of a thin chorion one cell layer thick, accompanied by the enlargement of the oocyte to 400–600 μm in diameter; and (3) differentiation of the crystalline PAS-positive yolk platelets in the peripheral cytoplasm. These three processes are followed by rapid vitellogenic growth, with the oocyte reaching 3.5 mm in diameter at the completion of vitellogenesis (Doroshov et al., 1991).

The increase in plasma concentrations of vitellogenin (our definition of the first stage of puberty in the female) is evident only after the completion of the first two morphological events. Concentration of vitellogenin continues to increase toward the end of oocyte growth and decreases before spawning. Synthesis of vitellogenin in sturgeon is readily stimulated by

treatment with estrogen at any age and in both sexes (Moberg et al., 1991a).

From purified fractions of sturgeon pituitary glands provided by H. Papkoff (University of California, Davis), we identified two fractions that appear to be functional analogues of the gonadotropins GTH I and GTH II in salmonids (Kawauchi et al., 1989; Swanson et al., 1989). We have designated our pituitary fractions sturgeon gonadotropin I (stGTH I) and sturgeon gonadotropin II (stGTH II), and we have developed radioimmunoassays to measure these two hormones (Moberg et al., 1991b). We think that stGTH I is responsible for inducing and maintaining vitellogenesis and stGTH II for regulating final ovarian maturation and ovulation. During reproductive maturation, a significant change occurs in the amount of the two stGTHs in the pituitary gland in both males and females (Figures 1 and 2). These data further support our earlier findings that the two stGTHs are involved in reproduction and that an increase in the pituitary content of these hormones correlates with the onset of puberty.

Role of Gonadotropin-Releasing Hormone

Because access to mature females is extremely limited, we used mature males and an analogue (GnRHa) of GnRH initially to define the role of GnRH in the secretion of the stGTHs. The analogue is similar in structure to sturgeon GnRH (Sherwood et al., 1991) and will induce spawning in sturgeon (Doroshov and Lutes, 1984; Fuji et al., 1989; Gontcharov et al., 1991). A single intramuscular injection of GnRHa induced secretion of stGTH I with only a marginal effect on stGTH II. This response was variable, apparently correlated with the plasma concentration of testosterone present before the injection of GnRHa (Moberg and Doroshov, 1992). When males were exposed to continuous stimulation from implanted pellets of GnRHa, there seemed to be a negative correlation between the plasma concentration of GnRHa and the amount of stGTHs secreted. Thus, in males, testosterone appears to enhance the ability of the pituitary gonadotrope to respond to GnRHa stimulation. However, continuous exposure to GnRH may result in down-regulation of the pituitary GnRH receptors, as occurs in goldfish (Peter, 1980).

In contrast to reproductively mature males, females in the previtellogenic stage of reproduction do not secrete stGTHs in response to administration of GnRHa (unpublished results). Because females at this stage of reproductive development still have relatively low pituitary concentrations of stGTHs, the failure to respond could reflect a lack of functional receptors or insufficient amounts of stGTHs for secretion. Because of the limited number of these valuable animals at fish farms, our initial studies have focused on younger animals to develop an understanding of the basic reproductive endocrinology.

Effects of Testosterone

Because the response to GnRHa seems to be positively influenced by testosterone, and testosterone stimulates accumulation of pituitary GTH in other species (Crim and Evans, 1983), we implanted silastic capsules containing 75 mg testosterone into 18-month old, sexually undifferentiated sturgeon (Pavlick et al., 1993). In fish sacrificed 21 days after implantation, the pituitary concentrations of stGTH I and stGTH II were significantly increased ($P < .01$) compared with concentrations in controls and in animals that had sham operations (implantations). Exogenous testosterone had no effect on plasma concentrations of either stGTH. Fish injected with 10 µg/kg of GnRHa 21 days after placement of testosterone implants also showed no increase in plasma of either stGTH. Pituitary concentrations of stGTH I and stGTH II were significantly increased ($P < .01$) in these fish compared with controls and with fish with sham implants. Exogenous testosterone apparently had a positive effect on the accumulation of stGTHs in the pituitaries of juvenile white sturgeon. However, GnRHa was ineffective in stimulating the secretion of these accumulated pituitary stGTHs.

In our continuing studies on the feedback effect of steroids on the gonadotrope of the immature sturgeon, we implanted silastic capsules containing testosterone into 24 month-old sturgeon and monitored the fish for several months to determine if prolonged treatment with testosterone might cause sustained increases in the amount of stGTHs in the pituitary gland and subsequent secretion into the circulation. In groups of sturgeon sacrificed 30, 60, or 90 days after implantation, pituitary concentrations of stGTH I were significantly greater ($P<.01$) in testosterone-treated fish than in controls. Pituitary concentrations of stGTH II were significantly higher ($P < .01$) in fish treated 60 or 90 days with testosterone than in controls. Exogenous testosterone had no effect on plasma concentrations of either stGTH. Sturgeon injected with 10 µg/kg of GnRHa 90 days after placement of testosterone implants also showed no change in plasma concentrations of stGTHs. These results indicate that long-term treatment with testosterone stimulates the accumulation of stGTHs in the pituitary gland in juvenile white sturgeon but does not affect basal or GnRHa-induced secretion of stGTH. The data also suggest that the inability of the pituitary gland to respond may be related to a lack of GnRH receptors or to the strong negative feedback of testosterone or some other endogenous inhibitor in prepubertal animal.

In the spring of 1994, previtellogenic females treated with 150 mg of testosterone (implants) also had higher levels of pituitary stGTHs than controls did, suggesting that the pituitary glands in adult fish are also affected by this strong positive feedback of testosterone. Testosterone had no effect on plasma concentrations of stGTH or on ovarian development.

Effect of Dopamine

In several species of fish (Copeland and Thomas, 1989), dopamine acts as an endogenous

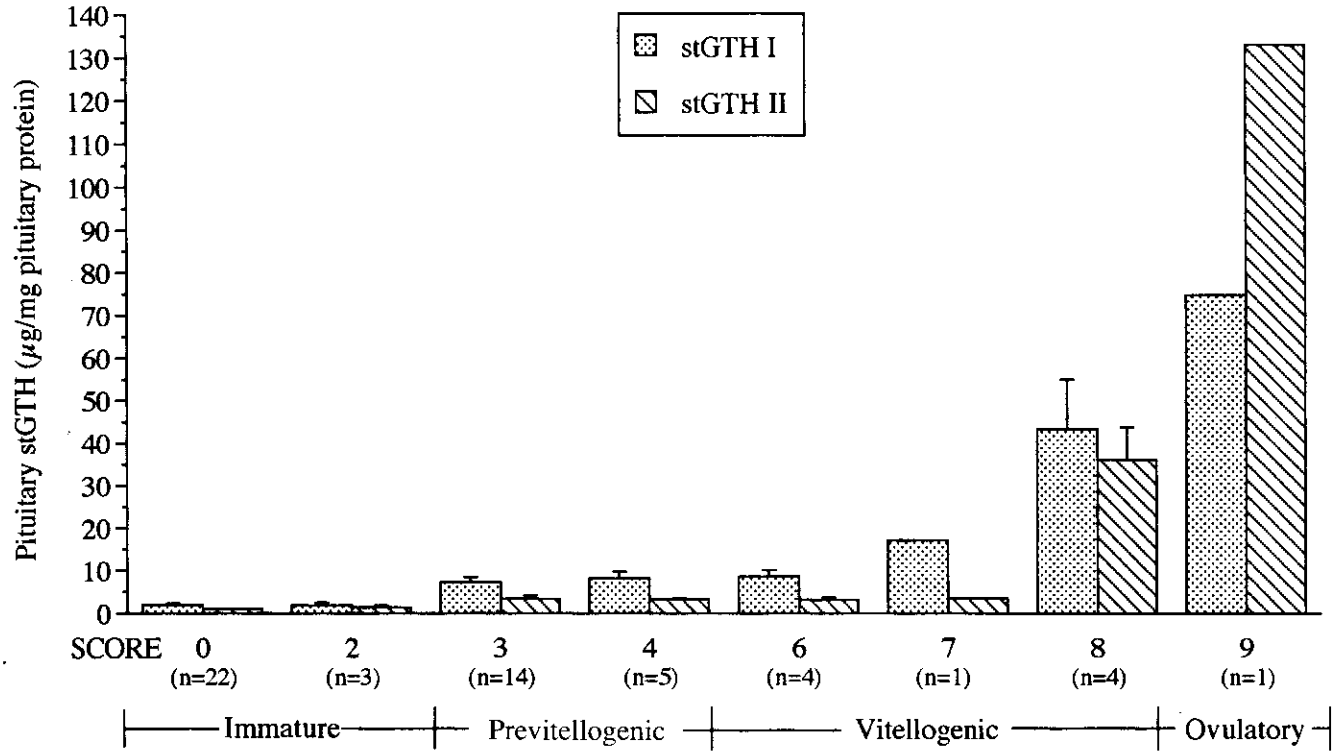

Figure 1. Mean (± standard error of the mean) pituitary concentrations of stGTH I and stGTH II in cultured female sturgeon during the immature (scores 0–2), previtellogenic (scores 3–4), vitellogenic (scores 6–8), and ovulatory (score 9) stages of reproductive development. Values in parentheses indicate the number of sturgeon per score.

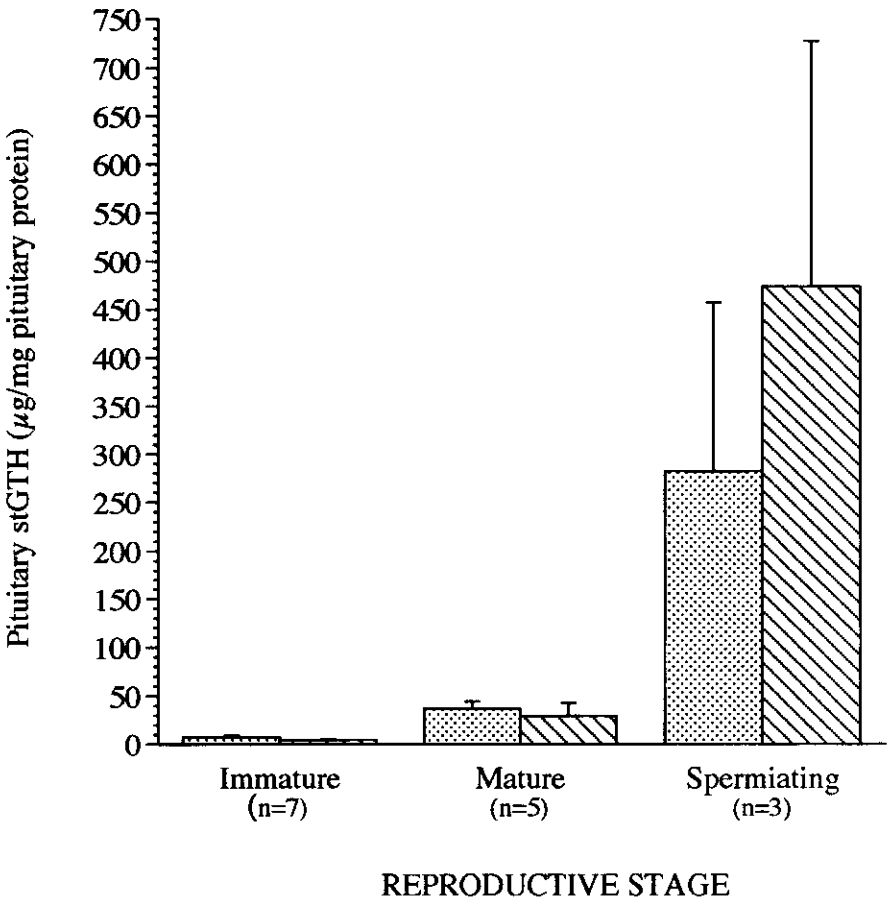

Figure 2. Mean (± standard error of the mean) pituitary concentrations of stGTH I and stGTH II in reproductively immature (gonial), mature (meiotic), and spermiating male sturgeon. Values in parentheses indicate the number of sturgeon per reproductive stage.

inhibitor of GnRH-induced pituitary secretion of GTH. In the silver eel, prepubertal blockage of gonadotropic function can be overcome by treating the fish with a combination of GnRHa and a dopamine antagonist, resulting in the stimulation of ovarian development (Dufour et al., 1988). In 1994 we determined if a comparable dopaminergic inhibition occurs in sturgeon. At time zero, groups of fish were injected with GnRHa (10 µg/kg) or an equivalent volume of saline. Six hours later, groups of fish were injected with dopamine (100 mg/kg) or an equivalent volume of vehicle. Blood samples were obtained at various times, and plasma concentrations of stGTH I and stGTH II were determined. Fish receiving GnRHa only had significantly higher ($P < .01$) concentrations of both stGTHs than fish receiving physiological saline (PS), dopamine, or a combination of GnRHa and dopamine (Figures 3 and 4). Two hours after administration, dopamine was effective in decreasing plasma concentrations of both stGTHs that had been increased by injection of GnRHa. Dopamine or PS alone did not alter plasma concentrations of either stGTH.

We also assessed the effects of the dopamine antagonist pimozide on mature male sturgeon during spermiation and on previtellogenic females. Initially, groups of fish were given either pimozide (5 mg/kg) or saline. Twelve hours later (zero time), groups were injected with GnRHa (10 µg/kg), pimozide (5 mg/kg), a combination of GnRHa and pimozide, or an equivalent volume of PS. Blood samples were obtained at various times and analyzed for both stGTHs. In mature males, pimozide potentiated secretion of both stGTHs when used in combination with GnRHa. Pimozide alone did not increase secretion of stGTH in these males. Treating previtellogenic females with pimozide, GnRHa, or a combination of pimozide and GnRHa, did not result in secretion of stGTH.

Seasonal and Environmental Influences

According to our field monitoring data, maturation of female sturgeon

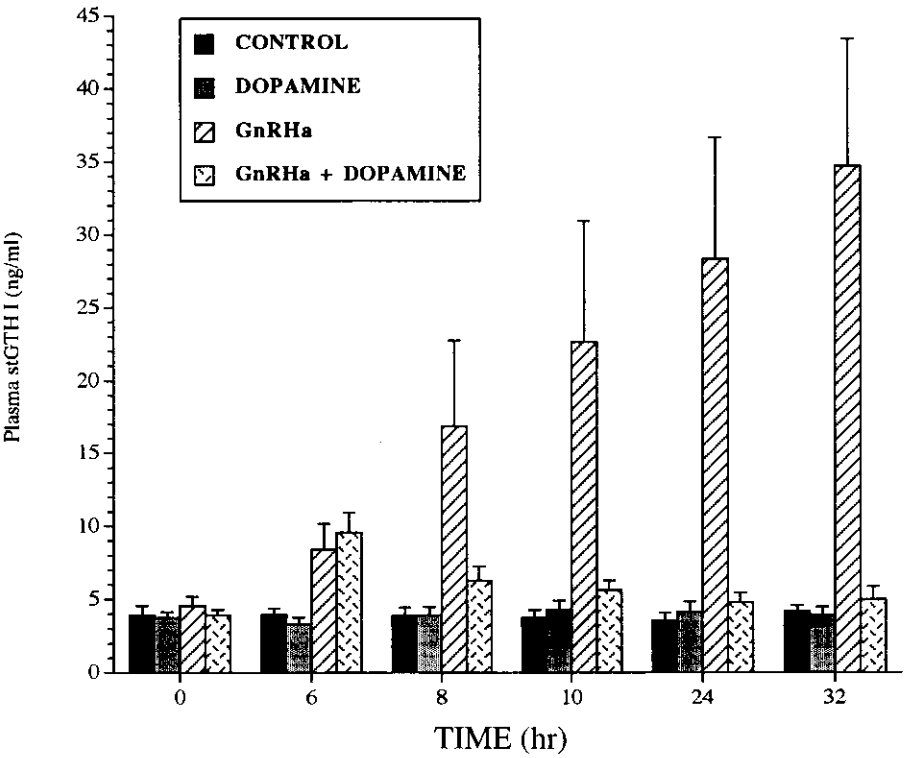

Figure 3. Effects of GnRHa (10 µg/kg, administered at 0 hr) and dopamine (100 mg/kg, administered at 6 hr) on plasma stGTH I (mean ± standard error of the mean); n = 8 for controls, h = 7 for other treatment groups.

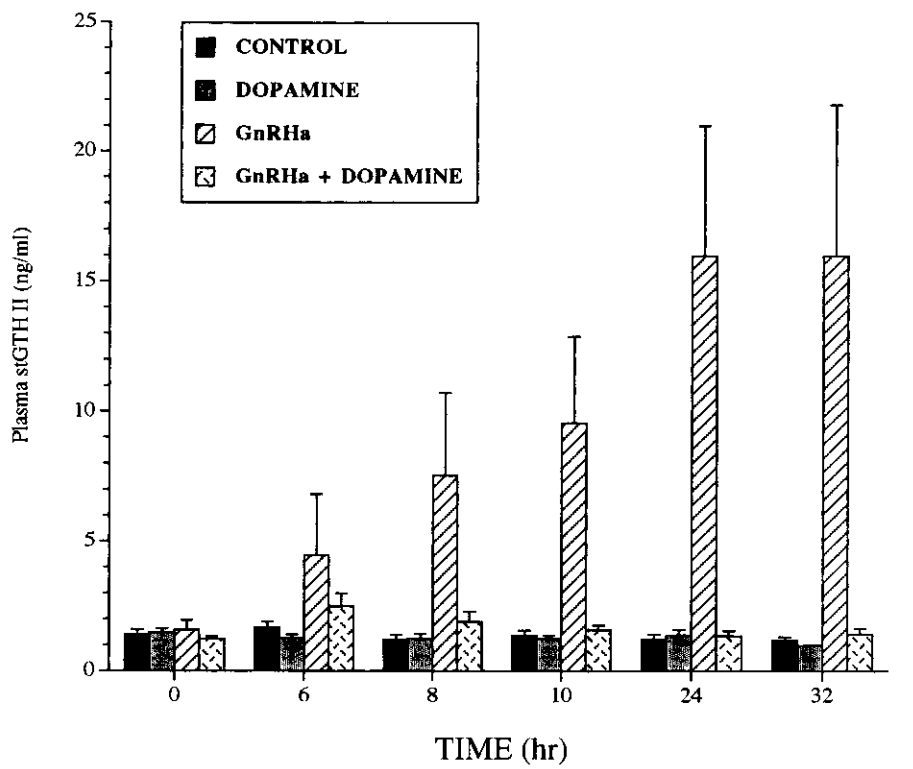

Figure 4. Effects of GnRHa (10 µg/kg, administered at 0 hr) and dopamine (100 mg/kg, administered at 6 hr) on plasma stGTH II (mean ± standard error of the mean); n = 8 for controls, h = 7 for other treatment groups.

is characterized by the following stages. In the fall, the granulosa and chorion layers differentiate without any visible yolk platelets. The following spring deposition of yolk platelets occurs along with a dramatic increase in yolk synthesis and oocyte growth that extends throughout the summer. By fall, some fish in captivity have black eggs (i.e., acquire melanin pigment granules), which are indicative of spawning the following spring. Those in which black eggs do not develop cease vitellogenesis during the winter and continue the process the following summer. Fish with black eggs enter the phase of final maturation the next spring when eggs polarize and appear ready for spawning. Unfortunately, hormonal stimulation of follicles in many of these fish does not lead to egg maturation. These data suggest strong seasonal and environmental influences.

We have been monitoring a group of females for several seasons by measuring plasma concentrations of hormones and gonadal development. Two years ago these females reached the black-egg stage and were injected with GnRHa and carp pituitary extract according to our standardized spawning procedure. Two of the fish did not ovulate, two became atretic, and the remainder ovulated. From these data, we were able to establish the normal endocrine changes that occur in female sturgeon as they approach spawning. Plasma concentrations of sex steroids (testosterone and estradiol) increased during vitellogenesis. The concentration of estradiol decreased after vitellogenesis, but levels of testosterone remained high. Concentrations of the two stGTHs were low during vitellogenesis but increased at spawning, particularly stGTH II. Ovulatory females had high plasma concentrations of progesterone. Preliminary observations on hormonal profiles and oocyte morphology indicated that seasonality (natural photoperiod and water temperature) plays a significant role in the ovarian cycle. Differentiation of the granulosa cell layer and deposition of the chorion occurred mostly during the late fall, coinciding with a significant increase in plasma levels of testosterone that occurred before the increase in levels of estradiol. The appearance of platelet yolk in the cytoplasm occurred only after several months (subsequent spring and summer) and coincided with a significant increase in plasma levels of estradiol.

We found that season also influences the gonadotropes' response to exogenous GnRHa (Figure 5). Males treated with GnRHa in August secreted little stGTHs, whereas those treated in December (just before spawning season) and in April (during spawning season) secreted significant amounts of stGTHs in response to injections of GnRHa.

During the first attempts of domestic sturgeon breeding in 1991 and 1992, we observed poor spawning and egg quality in domestic females and attributed this to elevated water temperature during the prespawning period. However, this assumption could not be confirmed because of the small number of gravid females available. During the spawning season of 1993, we conducted the first experimental observations on the effect of temperature on final ovarian maturation. Three groups of females with similar stages of ovarian development were subjected to three temperature treatments from December 1992 to June 1993 as follows: (1) constant temperature of 15°C, which is optimal for sturgeon spawning; (2) ambient seasonal temperature, ranging from 10°C in January to 15°C in April–June; and (3) constant 19°C temperature, which is similar to the temperature used for rearing sturgeon broodstocks at commercial farms. Fish were sampled monthly for state of egg development and plasma hormone profiles. Eggs collected by catheterization were incubated for 16–22 hours at temperatures of 16°C and 24°C to examine egg maturation in response to *in vitro* treatment with progesterone and sturgeon pituitary extracts. The females in a responsive stage have been spawned (injected with GnRHa and carp pituitary extract), and their egg quality (fertilization success and hatchability) has been evaluated.

Treatment 1 resulted in only one (20%) successful spawning, weak *in vitro* egg maturation response, and no ovulatory response in the majority of injected females. Treatment 2 resulted in successful spawnings in all fish and high fertilization and hatching success (80% and 50%, respectively). Treatment 3 resulted in rapid atresia in most fish and two unsuccessful spawnings.

Females in the second treatment group had a gradual decrease in levels of plasma estrogen; testosterone levels remained relatively high until spawning. Injection of GnRHa and carp pituitary extract produced an ovulatory surge in stGTH II and progesterone. Fish in treatment groups 1 and 3 had a rapid decline in both testosterone and estrogen before their eggs reached the responsive stage. Secretion of stGTH II and progesterone in response to stimulation with GnRHa and the maturational response of their oocytes to *in vitro* stimulation with progesterone and pituitary extract were significantly reduced, compared with fish in treatment group 2. This work has provided the first experimental evidence that white sturgeon females require winter water temperature (decrease of temperature to at least 10°C) for normal spawning in the spring and the production of good quality eggs. Excessively high or constant water temperatures held at spawning level, lead to major changes in the secretion of sex steroids by the follicular envelope and result in either developmental arrest or atretic changes in the oocyte.

Summary

Our findings allow us to make several assumptions about the endocrine control of reproduction in sturgeon. Like salmonids, sturgeon have two GTHs controlling reproduction. GnRHa stimulates secretion of stGTH in reproductively mature male sturgeon and induces spawning in mature females but does not stimulate secretion of stGTH in previtellogenic females. Dopamine

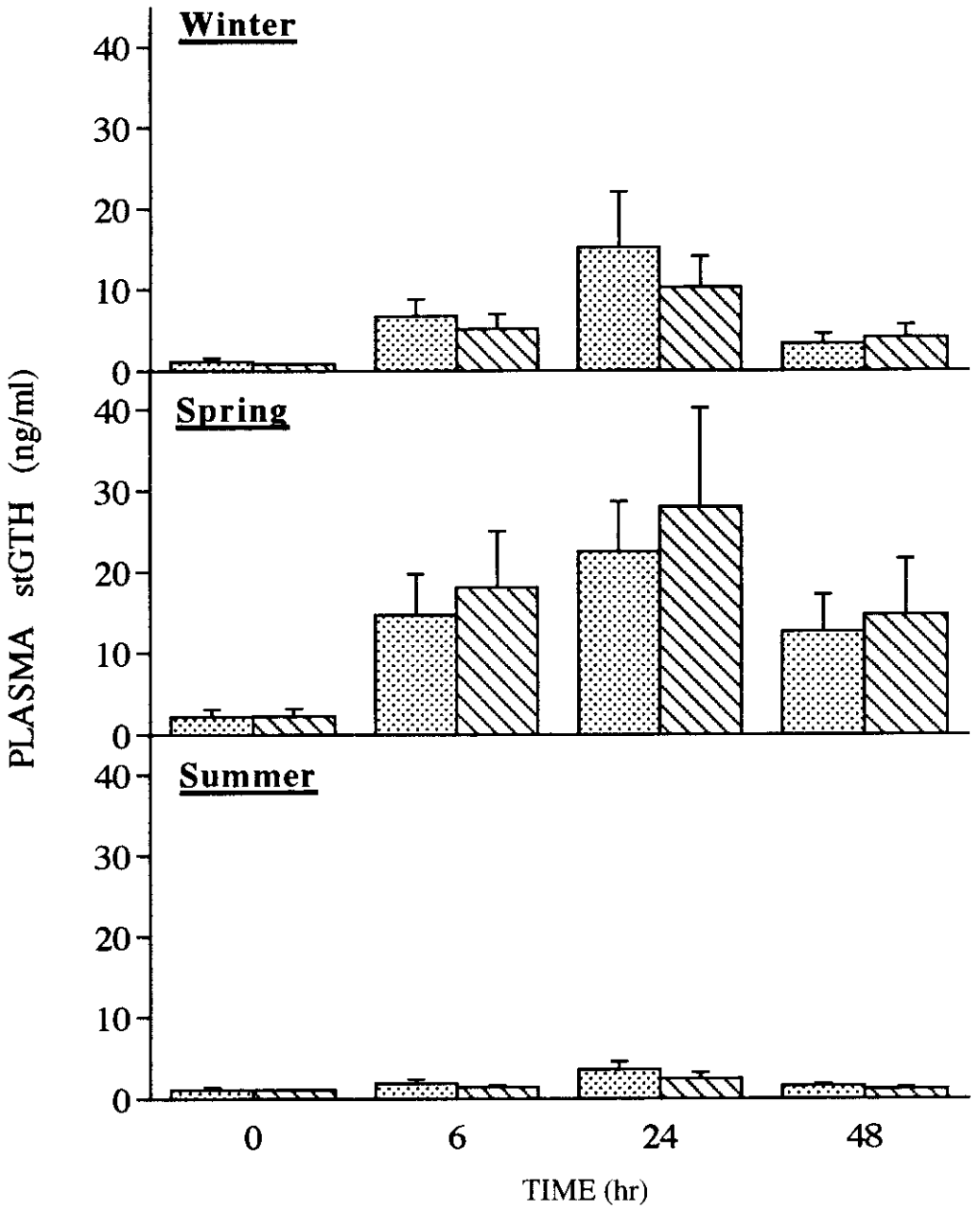

Figure 5. Seasonal effects of exogenous GnRHa (10 µg/kg, administered at 0 hr) on mean (± standard error of the mean) plasma concentrations of stGTHI and stGTH II at 0, 6, 24, and 48 hr after GnRHa injection in adult male sturgeon (n = 10).

inhibits secretion of stGTH in mature male sturgeon. The dopamine antagonist pimozide potentiates secretion of stGTH in spermiating males but has no effect on release of stGTH in previtellogenic females. Testosterone increases the pituitary concentration of stGTH in sexually undifferentiated sturgeon, but GnRHa does not stimulate the release of stGTHs in these fish. There is a seasonal influence on the responsiveness of the gonadotrope to GnRHa in males. Likewise, season influences egg maturation. Maturing females nearing spawning need to be exposed to water temperatures as low as 10°C to ensure successful spawning.

Collectively, these data offer some potential solutions to the problems facing the sturgeon aquaculture industry. We think our studies have shown that proper environmental control and defined endocrine treatments can have significant effects on sturgeon maturation and development, spawning success rates, and egg viability.

Cooperating Organizations
Hudson River Foundation
U.S. Fish & Wildlife Service
White Sturgeon Broodstock Research & Development Program

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Stimulation of Growth Hormone Gene Expression in Abalone: Applications for Acceleration of Growth and Improvement in Food-Conversion Efficiency and Resistance

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R/A-86
1991–93

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Our long-term goals are to use a molecular genetic approach to determine the nutritional, environmental, and genetic factors that regulate growth in abalone and other molluscan shellfish and to develop cost-effective applications that can be used to safely accelerate the growth rate and improve food-conversion efficiency and resistance to stress in abalone grown under conditions of commercial aquaculture operations. One of our major objectives was to develop the necessary molecular methods for this work with abalone. Another was to determine whether abalone brain cells produce the growth-accelerating hormone known as molluscan insulinlike peptide (MIP), and, if so, to establish a cDNA library from the abalone brain that would enable us to clone and characterize the abalone gene that codes for this hormone. Using these clones, we can determine the nutritional, environmental, and genetic factors that regulate the expression of this gene and thus accelerate growth. This strategy for stimulating production of the endogenous hormone should make it possible to accomplish these improvements without resorting to the undesirable (and possibly banned) use of additions of exogenous hormones or genetic manipulation of the DNA of the cultured stock.

Profitability of the abalone aquaculture industry and cost-effective production of sufficiently large seed for restoration of natural stocks currently are limited by the bottlenecks of slow growth, low food-conversion efficiency, and low resistance to stress during grow-out. In our previous Sea Grant research, we found that simply adding low concentrations of heterologous (mammalian) insulin and growth hormone (GH) to the seawater surrounding juvenile abalone increased growth rate and the efficiency of nutrient assimilation in the animals (Morse, 1981, 1984; Hooker and Morse, 1985). These initial observations have been confirmed by independent experiments with two other species of abalone in Japan (by Prof. Kawauchi; see Kawauchi and Yasuda, 1989) and Australia (by Prof. Hanna), and extended to work in oysters by Prof. Chen at the University of Maryland (Paynter and Chen, 1991; see Morse and Morse, 1991, for review). These results suggested to us that growth and nutrient assimilation in abalone and other molluscs are controlled by high-affinity receptors that recognize insulinlike and GH-like peptides and that the molluscs produce these growth-regulating peptide hormones endogenously.

The results of our project confirm that conclusion. We accomplished our objective by showing that abalone produce their own MIP. In collaboration with our colleagues in Holland (who first discovered MIP and showed that it is a growth hormone in a freshwater snail; see Smit et al., 1988; Geraerts et al., 1992), we found by immunohistochemical staining and electron microscopy that specific cells in the abalone brain produce large quantities of this peptide. Our discovery that the brain is the site of production also supports our working hypothesis that nutritional and environmental signals regulate growth through a series of switches controlled by cells of the central nervous system. This discovery brings us within reach of a means to determine the nutritional and environmental signals required to activate expression of the genes that code for the growth-accelerating hormones. The determination of these signals should make it possible to develop a cost-effective method to safely accelerate the growth of abalone and other shellfish in culture.

To accomplish our goal of cloning the abalone gene for MIP, we first had to develop new methods for purifying abalone mRNA (the gene product) and generating cDNA (the complementary copy of the gene). Methods developed for other species were useless with abalone and related marine shellfish because of the large amount of mucus in the extracts of abalone tissues. We found that the tight binding of the polyanionic proteoglycans of mucus to the mRNAs prevents efficient access by the enzyme known as reverse transcriptase, which is used to copy the mRNA molecules to produce the complementary cDNAs needed for cloning. We also found that homogenization of the abalone tissues in ice-cold guanidinium isothiocyanate, a potent chaotropic agent, allows easy extraction and purification of the mRNAs as full-length molecules free of the troublesome polyanionic contaminants. These new and improved methods yield high-purity mRNA from abalone tissues and can be used to produce the required full-length cDNA. Three papers describe these new methods: Groppe and Morse, 1993a, 1993b, 1994. The methods are widely applicable to the mucus-rich tissues of a large number of marine molluscs, finfish, and shellfish for which previously available methods were inadequate, and we expect these procedures to have a significant impact on research and development in marine biotechnology and aquaculture with many marine species.

Using these new methods, we extracted mRNA from the brains of growing abalone and used it to construct a library of the cloned abalone brain cDNA molecules. We

recently identified several cloned cDNAs in this library that apparently correspond to the abalone gene for MIP, and we are characterizing these clones.

The Abalone Farm, Inc. (Cayucos, California); Ab Lab, Inc. (Pt. Hueneme, California); The Cultured Abalone, Inc. (Santa Barbara, California); and The Santa Barbara Abalone Association have provided technical information, fast-growing abalone, and stocks of feed algae that have been useful in our research.

The basic research on the genes for growth-stimulating hormones and the regulation of these genes has been conducted in close collaboration with Prof. T. Chen (Center of Marine Biotechnology, University of Maryland), who is pursuing parallel studies with oysters, and with colleagues from abroad who have provided an exchange of essential gene sequence information and research personnel.

The international collaborations in this project have been particularly strong, and they continue to grow. Our work with colleagues at the Vrije Universiteit in Amsterdam, the Netherlands, has been most productive. A student researcher from Prof. Geraerts's research team worked in our laboratory for 10 months this past year, and Prof. Geraerts and two senior postdoctoral students who collaborated in the successful immunohistochemical detection of MIP in the abalone brain joined us for work during two shorter visits. Recently, a graduate student from our laboratory spent 2 weeks at the laboratory of our colleagues in Amsterdam, receiving advanced training in the techniques of immunohistochemistry and *in situ* hybridization adapted to the molluscan nervous system. In our collaboration with researchers in Taiwan and Japan, Dr. J.-L. Wu (Chairman of Zoology and Director of the Marine Biotechnology Initiative, Academia Sinica, Taiwan) and Prof. Kawauchi (Kitasato University, Japan) have provided peptide sequences useful for our work. We recently launched a joint effort with the Aquaculture Faculty in Galway, Ireland; this group has sent several researchers to our laboratory in the past, and the facility at Galway is using technologies we developed for their commercial production of both Irish and Japanese abalone species.

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Profs. J.-L. Wu and J.C.-M. Kuo

Cooperating Organizations

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Ab Lab, Inc., Pt. Hueneme, California
Academia Sinica
Aquaculture Faculty, University of Galway, Ireland
Center of Marine Biotechnology, University of Maryland, Baltimore
Kitasato University, Japan
National Taiwan University, Taipei
Santa Barbara Abalone Association, California
Taiwan National Program in Marine Biotechnology
The Cultured Abalone, Inc., Santa Barbara, California
Vrije Universiteit, Amsterdam, the Netherlands

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Development and Characterization of a High-Rate Biofilter for Recirculating Aquaculture Systems

University of California, Davis
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1991–94

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The overall objective of the project reported here was to develop a high-rate biological filter for removing ammonia from water used to culture fish, characterize the filter's operational parameters, and propose design guidelines. Specific objectives for the work included the following:

- Determine the range of possible water flow rates that could be treated with laboratory-scale trickling columns by using ammonium chloride and table sugar to simulate the presence of fish waste in the water.
- Determine the effect of oxygen concentration in the column on the rates of ammonia removal (nitrification) by using laboratory-scale columns with (1) table sugar and ammonium chloride and (2) real fish-tank water. Two types of filter medium were tested: randomly packed plastic rings and rigid cross-flow blocks.
- Test a prototype-sized trickling column for treatment of fish-tank effluents.
- Determine the maintenance requirements and devise cleaning methods for the trickling columns operating at high flow rates with fish-tank effluents.
- Propose design guidelines for high-rate trickling filters used in aquaculture.

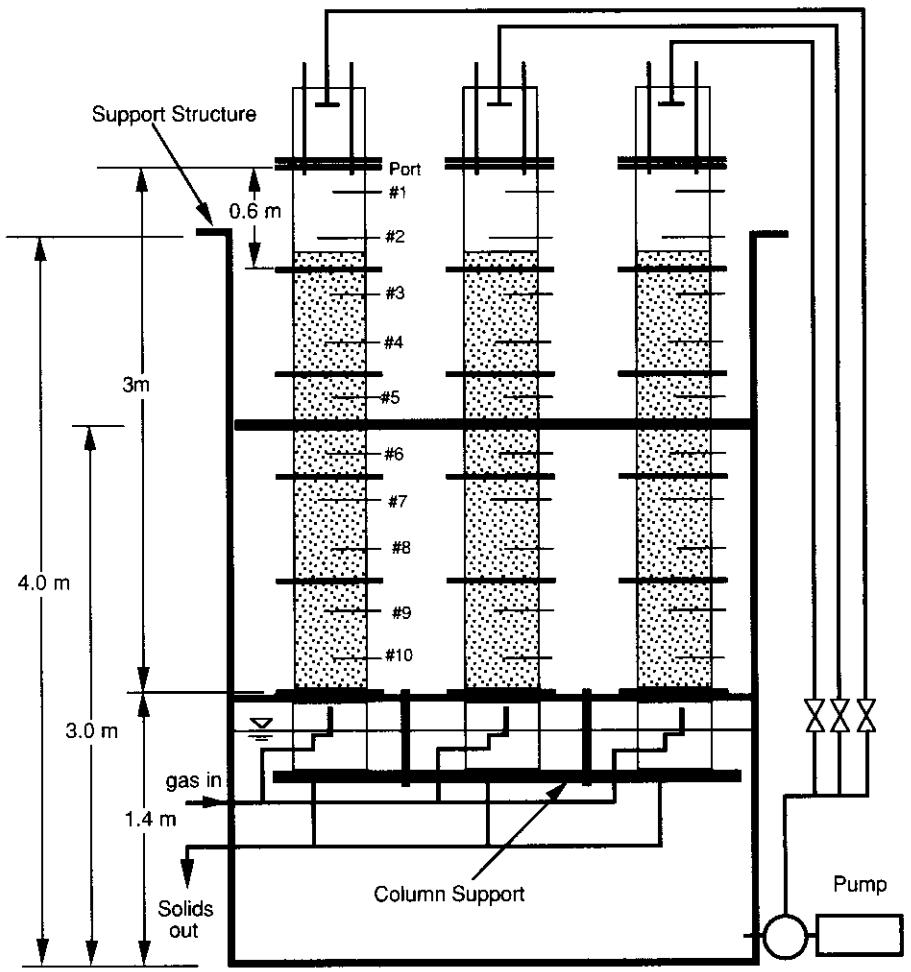

Figure 1. Schematic diagram of installation shows three laboratory-scale trickling columns.

Methods

Experimental work was carried out initially with three laboratory-scale columns 3 m high, 0.15 m in diameter, made from acrylic glass (Figure 1). A larger scale prototype column, 3 m high, 0.45 m in diameter, made from fiberglass, was constructed and tested. The laboratory-scale columns were tested with two different types of packing medium: randomly packed plastic cylinders (1.6 cm in diameter × 1.6 cm long Pall™ rings) with a specific surface area of 341 $m^2\ m^{-3}$, and rigid cross-flow medium made from corrugated sheets (Biostrata™) with a specific surface area of 223 $m^2\ m^{-3}$. The prototype column was tested with randomly packed medium (1.6 cm in diameter × 1.6 cm long Bio Barrels™) with a specific surface area of 344 $m^2\ m^{-3}$.

The trickling columns were installed over a 3 m^3 reservoir (Figure 1). Water was pumped to the top of the columns through flow meters, flowed down the columns, and was discharged back into the reservoir. Gas (air, a mixture of air and nitrogen, or a mixture of air and oxygen) was introduced at the base of the columns through flow meters. The columns were backwashed by filling to approximately 1.2 m with water and injecting pulses of air at the bottom of the columns by using a solenoid valve. Air pulses caused significant turbulence, lifting and agitating the packing medium. Pulsing was carried out for several minutes, and then the water in the column was allowed to settle, and the high-solids backwash was drained and removed from the system.

The columns were tested initially with a "synthetic fish waste" made from sucrose (table sugar) and ammonium chloride mixed in a mass ratio of 4.7:1. Sucrose was added to simulate the presence of dissolved organic material in fish wastes and the associated biochemical oxygen demand the material represents. The ammonium chloride served as the source of ammonia for the nitrifying bacteria in the biofilter. The mixture of sugar and ammonium chloride was diluted in water to prepare a stock solution that was stored in a refrigerator at 4°C and introduced into the tank with a metering pump. The rate of injection of synthetic fish waste was selected to match the rate of nitrification taking place in the columns and to maintain an approximately constant concentration of total ammonia nitrogen (TAN) in the system.

Water temperature in the system was maintained at 25°C throughout the tests. Alkalinity was maintained at more than 1 meq l^{-1} and pH at more than 7.0 by water exchanges and by the addition of sodium bicarbonate. Dechlorinated tap water was used to replace water removed from the system. Alkalinity of the tap water was approximately 3 meq/l.

Determinations of nitrification rates were based on mass balance calculations: of TAN for the tests with synthetic fish waste, and of nitrite and nitrate for the tests with fish. The use of a nitrite and nitrate mass balance for the fish tests was necessary because the rate of addition of ammonia to the system (from the fish) was not known. According to standard practice, nitrification rates are expressed per unit of medium surface area.

Results

The results are presented in relation to the objectives mentioned in the introduction.

Range of flow rates. Initial tests with the laboratory-scale columns were carried out with randomly packed medium (1.6 cm Pall™ rings) at hydraulic loading rates of 188, 1879, and 3758 $m^3 m^{-2} d^{-1}$, corresponding to 2, 20, and 40 l/min for the columns. The TAN concentration in the system was maintained at approximately 2 mg/l by the addition of synthetic fish waste as described. At the lowest flow rate tested, almost no bacterial slime accumulated on the surface of the medium in the column. The rings remained clear for the duration of the experiment (approximately 6 months). The only biological activity observed in the low-flow column was the proliferation of flies and the presence of their larvae. Heavy growth of slime (biofilm) was observed in the columns at the other two flow rates tested. The rings in the two high-flow rate columns (20 and 40/l min) became covered with bacterial slime and required frequent backwashing. The problem was particularly severe in the column with the highest flow rate, where thorough backwashing was difficult to achieve and the air and water flows tended to be restricted.

Nitrification rates were highly variable, with extended periods of very low rates. These periods of low nitrification became more frequent and extensive toward the later part of the experiment and presumably were due to excessive growth of biofilm and the associated reduction in surface area and contact time in the columns caused by clogging. The average nitrification rate was 0.2 g $m^{-2} d^{-1}$, which was comparable to that reported previously for trickling filters used in aquaculture (Miller and Libey, 1985; Rogers and Klemetson, 1985). The severe clogging problems in the column with the highest flow rate led to the selection of a hydraulic loading rate of 2255 $m^3 m^{-2} d^{-1}$ (24 l/min) for all other tests.

Effect of oxygen concentration on nitrification rates. The tests to determine the effect of oxygen concentration on nitrification rates with synthetic fish waste were repeated for the two types of medium already mentioned, at a TAN concentration of approximately 2 mg/l, and at concentrations of dissolved oxygen approximating 50, 100, 150, and 200% of saturation (actual average values were 56, 98, 144, and 203% saturation). Nitrification rates for the rigid cross-flow medium were determined also for a TAN concentration of 5 mg/l. In all cases, the system was allowed to stabilize for at least 1 week at a particular concentration of dissolved oxygen before nitrification rates were determined. The various concentrations of dissolved oxygen were achieved by injecting into the packed column a mixture of air and nitrogen gas or a mixture of air and pure oxygen. Serious clogging problems occurred frequently during the experiments, and backwashing was carried out at least once per day in some cases. Backwashing was required more often at 150 and 200% saturations of dissolved oxygen than at the 50% level.

Nitrification rates for the randomly packed medium and for the rigid cross-flow medium are shown in Figure 2 for the different concentrations of dissolved oxygen tested at a concentration of ammonia of less than 2 mg/l TAN. For the randomly packed medium, nitrification rates increased as the concentration of dissolved oxygen increased, with the highest rates occurring at 200% saturation. Statistical analysis indicated that the nitrification rate at 50% dissolved oxygen saturation was significantly different from the rates at the other oxygen levels but that there were no significant differences among the rates at 100, 150, and 200% saturation. The lower nitrification rate in the randomly packed medium for 50% dissolved oxygen saturation is likely due to an oxygen limitation caused by low availability of oxygen to the nitrifying organisms.

There was no significant difference among nitrification rates for the rigid cross-flow medium, suggesting that dissolved oxygen was not a factor limiting nitrification for the conditions found in the system. This is likely due to the more effective transfer of oxygen from the gas phase into the water and biofilm because of the absence of clogging and the presence of a thinner biofilm than in randomly packed medium. The rate of nitrification in the rigid cross-flow medium was consistently lower than for the randomly packed medium (Figure 2).

Nitrification rates with rigid cross-flow medium at a TAN level of approximately 5 mg/l were higher

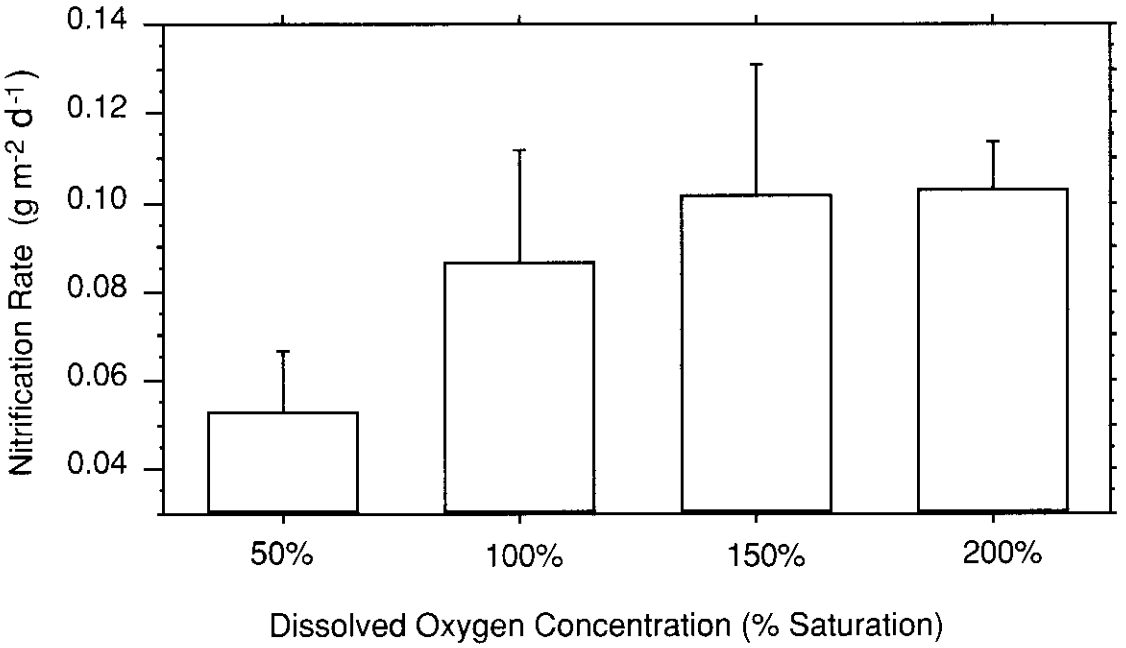

Figure 2. Nitrification rates obtained in the laboratory-scale columns filled with a randomly packed medium (1.6 cm Pall™ rings) at a total ammonia nitrogen concentration of approximately 2 mg/l.

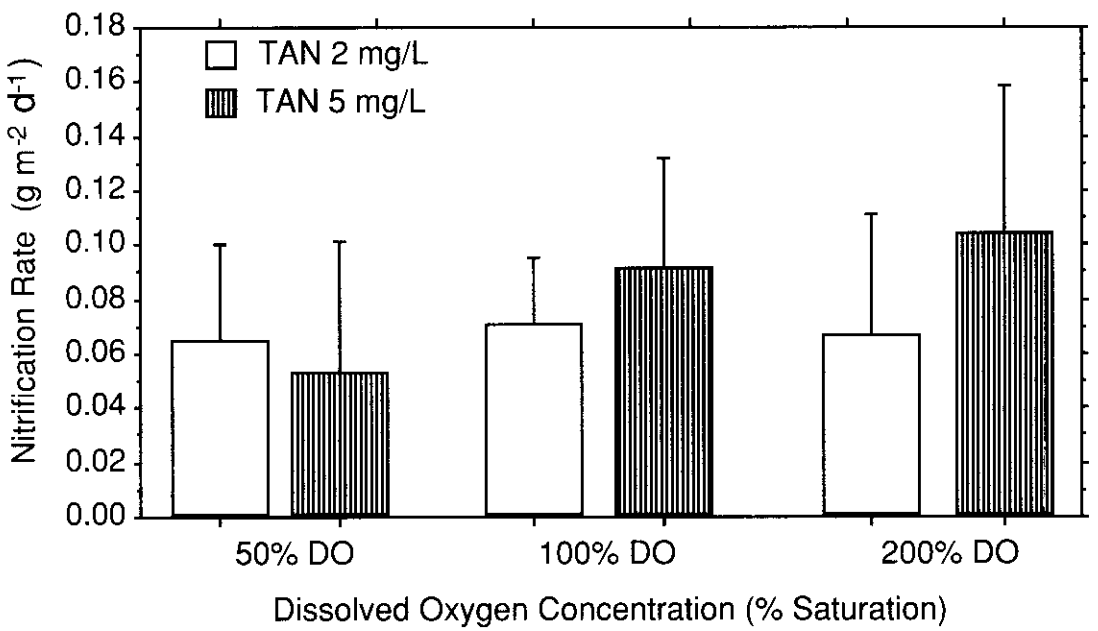

Figure 3. Nitrification rates in the laboratory-scale columns with rigid cross-flow medium (Biostrata ™) at two ammonia concentrations, 2 and 5 mg/l total ammonia nitrogen (TAN).

than those at 2 mg/l TAN (Figure 3). Statistical analysis showed a significant difference between the 2 and 5 mg/l TAN rates at 200% dissolved oxygen saturation. These results suggest that concentration of ammonia may be the factor limiting nitrification rates at low TAN concentration (less than 2 mg/l). At the slightly higher concentration of 5 mg/l TAN, concentration of dissolved oxygen apparently limits the rate of nitrification.

Tests with fish were done with the rigid cross-flow medium by placing approximately 25 kg of channel catfish in the 3-m^3 reservoir and feeding to approximately 1% per day (maximum feeding rate was 250 g/day distributed in three feedings). The packed column was the only water treatment used in the system. Approximately 5% of the system's water volume was replaced daily because of accumulation of solids and decreases in alkalinity and pH. TAN remained at less than 0.4 mg/l throughout the fish trials.

Nitrification rates in the fish trials were much higher than those for synthetic fish wastes under comparable hydraulic loading and oxygen

concentration. Rates with fish waste ranged from 0.53–0.30 g m^{-2} d^{-1} for oxygen levels of 50 and 200% dissolved oxygen saturation (compared with 0.07 g m^{-2} d^{-1} for synthetic fish waste and TAN less than 2 mg/l). Contrary to the results with synthetic fish wastes, nitrification rates with real fish wastes tended to decrease as the concentration of dissolved oxygen increased, although there was no significant difference among the rates at the various levels of dissolved oxygen. Rates were comparable to those reported previously by Miller and Libey (1985) (0.14–0.25 g m^{-2} d^{-1}) for channel catfish and by Bovendeur and coworkers (1987) (0.36 g m^{-2} d^{-1}) for African catfish. The TAN concentrations remained low for all concentrations of dissolved oxygen (mean values of 0.22, 0.19, and 0.12 mg/l TAN for 50, 100, and 200% dissolved oxygen saturations, respectively) and suggest that at these low TAN concentrations, nitrification rates are limited by the concentration of ammonia not the concentration of oxygen. The results also suggest that the full nitrification capacity of the filters was not reached because of the limited availability of ammonia. Rates of production of ammonia in the system were limited by the feeding rate of the fish and by the buildup of solids in the system.

Tests of the prototype column. The prototype column (3.0 m tall, 0.45 m in diameter) was filled to a depth of 1.8 m with 1.6 cm Bio Barrels™ and was tested with both synthetic fish waste and fish-tank water. Backwashing procedures developed for the laboratory-scale columns were used and were satisfactory. The column was tested at 100% dissolved oxygen saturation only. The rate of nitrification in the prototype column was approximately twice as high as that in the laboratory-scale columns under similar conditions (0.16 vs. 0.07 g m^{-2} d^{-1}). Nitrification rates obtained with fish tank water were approximately 0.22 g m^{-2} d^{-1}, and the concentration of ammonia remained low (less than 0.2 mg/l) for the duration of the tests. As was the case with fish waste with the laboratory-scale columns, the full nitrification capacity of the column was probably not reached because of the limited production of ammonia by the fish. Further testing of the column is under way to determine nitrification rates when the concentrations of ammonia are higher.

Cleaning methods and maintenance. Heavy biofouling of the columns occurred with both synthetic and real fish waste. Without maintenance and column backwashing, the randomly packed columns clogged up restricting the flow of air and water. Clogging did not occur in columns with the rigid cross-flow medium, which did not require backwashing but had lower nitrification rates. Backwashing was carried out by filling the columns with water to a depth of approximately 1.2 m and injecting pulses of air at the bottom of the columns. The process was continued for several minutes, and then the material in the column was allowed to settle and was flushed from the system. The total volume of sludge removed in each backwashing was approximately 0.015 m^3 for each of the laboratory-scale columns and 0.16 m^3 for the prototype column. The concentration of suspended solids in the backwash was as much as 3700 mg/l. Backwashing was repeated daily or less often.

Design guidelines. The depth of the medium in all the columns tested was approximately 1.8 m. At this depth, and for the hydraulic and gas flow rates used in the experiments, columns were effective both as nitrifying biofilters and as packed-column aerators (Hackney and Colt, 1982; Colt and Bouck, 1984). Initial tests were carried out at hydraulic loading rates of 188, 1879, and 3758 m^3 m^{-2} d^{-1}. Columns tended to clog at the high hydraulic loading rate, and a rate no higher than 2255 m^3 m^{-2} d^{-1} is recommended. At this rate, and for ammonia concentrations less than 0.4 mg/l TAN, nitrification rates between 0.30 and 0.53 g m^{-2} d^{-1} have been obtained in trials with catfish waste at 25°C in laboratory-scale columns with rigid cross-flow medium. In tests with a prototype-scale column with randomly packed medium, nitrification rates were approximately 0.22 g m^{-2} d^{-1}.

Nitrification rates in all the fish-tank trials were limited by the availability of ammonia produced by fish, and ammonia concentration remained low in the system. Higher nitrification rates are possible at higher concentrations of ammonia, as indicated in trials with synthetic fish wastes. The effect of the concentration of dissolved oxygen on the rate of nitrification depended on the type of medium used and on the concentration of ammonia. For the rigid cross-flow medium at ammonia concentrations of approximately 2 mg/l TAN, the concentration of dissolved oxygen did not affect nitrification rates, whereas at a TAN concentration of 5 mg/l, the rate of nitrification increased as the concentration of dissolved oxygen increased. In trials with fish waste and rigid cross-flow medium and TAN concentrations less than 0.4 mg/l, increasing the concentration of dissolved oxygen did not result in improvements in nitrification rate. For the randomly packed medium at a TAN concentration of approximately 2 mg/l, a dissolved oxygen concentration of 50% of saturation resulted in significantly lower nitrification rates than the rates for 100, 150, and 200% saturation in tests with synthetic fish waste.

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Fisheries

Edible Films for Preventing Loss of Quality in Frozen Fish

University of California, Davis
R/F-139
1990–93

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Fish products lose quality when moisture is lost to the environment or oxygen diffuses into the fish flesh and enhances the oxidation of lipids. These undesirable changes lead to deterioration of texture, flavor, color, and odor. The result is a reduction of shelf life and the inability of the U.S. seafood industry to meet the growing demand for high-quality products.

To a great extent, frozen storage prevents or decreases undesirable changes and maintains the quality of fresh fish. However, although the rates of dehydration and lipid oxidation are slower in frozen fish than in fresh fish, these processes still occur. Thus, the frozen fish industry encounters problems in supplying quality products.

Invisible, edible films made from natural ingredients and formed on the surfaces of fish may be the means of providing an ample amount of high-quality frozen fish products. Edible coatings can prevent quality changes in foods by acting as barriers that control transfer of moisture, uptake of oxygen, and loss of volatile odors and flavors. Edible films can be made from proteins (e.g., gelatin, soy protein, casein, whey protein, zein), polysaccharides (e.g., alginate, pectin, cellulose, starch), lipids (e..g., waxes, acetylated monoglycerides, surfactants), or a combination of these materials (Kester and Fennema, 1986). Proteins have not been investigated as extensively as other biopolymers for the creation of edible films (Kester and Fennema, 1986). However, milk casein has been used to make edible emulsion films that were good moisture barriers (Krochta, 1990). The lipid material in the films provided good resistance to transfer of moisture; the casein provided good structural cohesion, bound the films to wet surfaces, and reduced the films' waxy appearance. Casein-lipid emulsion films have been successfully applied to fruit products to reduce moisture loss (Krochta et al., 1990; Krochta, 1991; Avena-Bustillos et al., 1993, 1994). The structure and numerous functional groups of proteins offer a wide choice of possible manipulations to achieve edible films with desirable mass-transfer characteristics. As a result, several opportunities exist for use of proteins for edible films, including on frozen fish.

Project Goals

The overall goal of this project was to develop films based on milk casein, milk whey protein, and other proteins that could be formed on the surfaces of frozen fish as coatings to prevent quality deterioration due to loss of moisture and oxidation of lipids. This was divided into three subgoals: (1) development and testing of stand-alone films formed from casein, whey protein, and soy protein alone, or in combination with acetylated monoglycerides or other lipids; (2) preliminary testing of film coatings on ice and on a model gel under freezing temperatures; and (3) evaluation on fish of the most promising film coating.

Evaluation of Stand-Alone Films

Excellent films were formed from casein, whey protein, and soy protein, either alone or in combination with acetylated monoglycerides or other lipids. The addition of lipids to protein films in an emulsion system decreased the permeability of the films to moisture and reduced many of the problems encountered when lipids alone are used. Table 1 contains data on moisture and oxygen permeability for these films.

Casein films. All forms of milk casein formed edible films, because of the characteristic random coil of the protein and the ability to form extensive intermolecular bonds. These films were transparent, flexible, soluble in water, and bland tasting. Edible plasticizers such as glycerin improved the flexibility of the film but were not necessary for handling. When plasticizer was added to increase flexibility, magnesium and calcium caseinate films had similar permeabilities; magnesium caseinate films provided the best barrier to moisture loss (Table 1). Films made from other forms of plasticized casein (micellar, rennet, sodium and potassium) had higher moisture permeabilities than films containing magnesium caseinate; films containing rennet had the highest permeability. Unplasticized sodium caseinate films had lower permeabilities than all the plasticized caseinate films. Treating a sodium caseinate film with calcium ascorbate buffer solution at the isoelectric point of casein made the film insoluble and reduced the film's moisture permeability (Table 1). Films formed from emulsions of sodium caseinate with C12 to C18 fatty acids, fatty alcohols, paraffin, carnauba, and beeswax had significantly reduced moisture permeability; beeswax had the greatest effect (Table 1). Caseinate films were also excellent barriers to oxygen (Table 1).

Whey protein films. Films made from isolates of whey protein were produced by heating 8–12% solutions of whey protein to 75°–100°C to denature the proteins. These films were extremely brittle and required the addition of plasticizer to form intact films. Films made from whey protein were insoluble in water because of the covalent disulfide bonds formed during the heat treatment. The moisture permeability of these films was higher than that of casein films; this was attributed to the amount of plasticizer needed to counteract brittleness (Table 1). As

Table 1. Water Vapor and Oxygen Permeability of Protein-Based Edible Films

Film Composition	Water Vapor Permeability ($g \cdot mm/m^2 \cdot d \cdot kPa$)	Test Conditions for Water Vapor Permeability Temperature (°C)	Relative Humidity Gradient (%)	Oxygen Permeability[a] ($cm^3 \cdot \mu m/m^2 \cdot d \cdot kPa$)
Magnesium caseinate:glycerin (4:1)[b]	43.9	25	0/77	-
Rennet casein:glycerin (4:1)[b]	56.0	25	0/77	-
Sodium caseinate[c]	36.7	25	0/81	-
Buffer-treated sodium caseinate (pH 4.6)[c]	20.9	25	0/86	-
Sodium caseinate:lauric acid (4:1)[b]	9.6	25	0/92	-
Sodium caseinate:acetylated monoglyceride (1:4)[c]	15.8	25	0/84	-
Calcium caseinate:beeswax (1.7:1)[c]	3.6	25	0/97	-
Sodium caseinate:sorbitol (2.3:1) [f]	-			3.2
Whey protein isolate:sorbitol (1.6:1)[d]	62.0	25	0/79	-
Whey protein isolate:beeswax:sorbitol (3.5:1.8:1)[e]	5.3	25	0/98	-
Whey protein isolate:sorbitol (2.3:1) [f]	-			4.3
Whey protein isolate:sorbitol (3.5:1) [f]	-			2.6
Soy protein isolate:glycerin (5:1), unheated[g]	58.9	27	0/68	-
Soy protein isolate:glycerin (5:1), heated[g]	44.4	27	0/70	-

[a]Oxygen permeability was measured at 23°C and 50% relative humidity.
[b]Ho, 1992; [c]Avena-Bustillos and Krochta, 1993; [d]McHugh et al., 1994; [e]McHugh and Krochta, 1994a; [f]McHugh and Krochta, 1994b; [g]Stuchell and Krochta, 1994
Dashes = not tested.

with films based on casein, those formed from emulsions of whey protein isolate and lipids had significantly lower moisture permeabilities (Table 1). Films made with whey protein were excellent barriers to oxygen; oxygen permeability was similar to that of caseinate films.

Soy protein films. Soy protein isolate is a complex mixture of proteins with widely different molecular properties. The majority of soy proteins are globular storage proteins with a quarternary structure. Heating the film-forming solution denatured the proteins and allowed possible disulfide interchange. Films made with soy protein isolate were extremely brittle and required the addition of a plasticizer such as glycerin to form intact films. Films made from Supro 620, a commercially available soy protein isolate, were quite transparent, had a shiny surface, and were bland tasting. The moisture permeability of films made from heated solutions was lower than that of films made from unheated solutions (Table 1), suggesting a more dense arrangement of the protein chains in the finished film. Treatment of the film solution with horseradish peroxidase to enzymatically cross-link the protein did not significantly improve the ability of finished films to prevent loss of moisture.

Preliminary Testing of Edible Film Coatings

Two phases of preliminary testing of edible film coatings for use on frozen fish were accomplished. These tests were performed to determine the optimal formulation for coatings used to prevent loss of moisture under freezing conditions.

In the first phase, dishes of ice were coated with emulsions of calcium caseinate (Alanate 310) and acetylated monoglycerides (Myvacet 5-07, 7-00, and 9-00) to examine the effect of (1) the total amount of solids in the solution, (2) the ratio of acetylated monoglyceride to protein, and (3) the type of acetylated monoglyceride in the emulsion. We found that the total amount of solids in the emulsion played the most important role in preventing loss of moisture. Increasing the percentage of solids in the coating resulted in reduced loss. The coating containing 20% solids reduced moisture loss from ice by approximately 60% compared with uncoated ice. The ratio of protein to acetylated monoglyceride had a small effect on moisture loss (lower ratio of protein to acetylated monoglyceride resulted in decreased moisture loss), and the type of acetylated monoglyceride had no significant effect.

In the second phase of preliminary testing, we used a model gellike surface composed of 1% xanthan gum/locust bean gum mixture (Kelgum) and 2% starch. The gel was heated and then formed into 35-mm round by 10-mm high cylinders, which maintained their shape under freezing and thawing conditions. The cylinders were coated by dipping them in calcium caseinate, sodium caseinate, whey protein, soy protein, and gelatin solutions alone, or in emulsions of these proteins with acetylated monoglycerides (Myvacet 9-08 and 9-45), and then were frozen to –23°C. After freezing, some of the gels were sprayed with acetylated monoglycerides to form a bilayer coating. Weight loss was monitored over a 10-week period. Results showed that moisture loss with the bilayer coatings and acetylated monoglycerides alone was significantly lower than that with protein and protein emulsion coatings. Among the proteins tested, whey protein-based coatings were the best barriers to loss of moisture.

The Effectiveness of Edible Films as Coatings on Frozen Salmon

The effectiveness of edible film coatings on frozen fish was determined by using silver salmon (*Oncorhynchus kisutch*) coated with a casein-based emulsion and king salmon (*Oncorhynchus tshawytscha)* coated with emulsions containing whey protein isolate.

Testing of silver salmon. Emulsions containing acetylated monoglycerides (Myvacet 5-07) and calcium caseinate (Alanate 310) (9:1), with 0.5% added hydroxypropyl methylcellulose to increase viscosity, were used to coat pieces of silver salmon. Additional treatments included no coating (control), vacuum packaging with Cryovac film, and coating with Myvacet 5-07 alone. The fish was stored at –23°C for 10 weeks and evaluated for moisture loss and oxidation of lipids.

All the coated samples lost moisture at a reduced rate compared with the uncoated control, which lost 15.7% moisture during the study. The samples vacuum packaged in Cryovac film lost no moisture. Loss of moisture with the other coatings was 2.7% with Myvacet 5-07 alone, 12.0% with an emulsion containing 20% total solids, and 13.5% with an emulsion containing 10% total solids. The emulsion coatings were less effective as moisture barriers than the pure lipid alone, perhaps because of the development of a porous structure in the dried emulsion coatings. The lipid coating, however, was less flexible than the emulsion coatings, and cracks formed in it during frozen storage.

Lipid oxidation was assayed by using three different methods: (1) determination of peroxide value, (2) 2-thiobarbituric acid test, and (3) headspace gas chromatographic analysis for pentane. The peroxide values of the control samples began increasing at 6 weeks of storage and continued to increase throughout the 10 weeks of the study. The pattern of increase in peroxide values was essentially the same for control samples and samples coated with emulsion. Again, this may have been due to the porous structure of the emulsion coatings. The peroxide values of the lipid-coated samples were always lower than those of the emulsion-coated samples. The peroxide values of vacuum-packaged samples did not increase during the 10-week testing period. The thiobarbituric acid test showed similar trends for control, emulsion-coated and lipid-coated samples. Gas chromatography was not appropriate for this study; every coating interfered with the estimation of pentane by preventing pentane evaporation from the samples during storage.

Testing of king salmon. On the basis of results of the tests with model gels, nine coatings were applied to pieces of king salmon: (1) a spray of a 10% solution of whey protein isolate, (2) a spray of a 10% solution of whey protein isolate followed by a spray of a 5% solution of antioxidant (2.5% ascorbic acid and 2.5% citric acid), (3) a spray of an emulsion containing 10% whey protein isolate and 10% Myvacet 9-08, (4) a spray of an emulsion containing 10% whey protein isolate and 10% Myvacet 9-45, (5) a spray of a 10% solution of whey protein isolate followed by a spray of Myvacet 9-08; (6) a dusting with a powder of whey protein isolate followed by a spray of Myvacet 9-08, (7) a dusting with a powder of whey protein isolate followed by a spray of Myvacet 9-45, (8) a spray of Myvacet 9-08 alone, and (9) a spray of Myvacet 9-45 alone.

Table 2 gives the weight of the coatings applied. Uncoated control samples were also prepared. The samples were stored at –23°C. Five samples per treatment were removed after 3, 5, 7, 9, and 11 weeks of storage. The samples were weighed to determine total weight loss, and then the lipids were extracted and tested for lipid oxidation. Moisture loss was calculated from the weight loss of the stored sample. Lipid oxidation was determined by using the peroxide value, which was the most useful method for estimating the degree of lipid oxidation in the silver salmon testing. We found no statistically significant differences between the two acetylated monoglycerides used, so results are reported for Myvacet 9-08 only. Moisture loss and peroxide values represent the average of five measurements for each treatment.

Figure 1 shows the relative moisture content of the fish samples over the 11 weeks of study. The moisture losses of samples coated with whey protein isolate alone or with emulsions containing whey protein isolate were comparable at all sampling times to losses from uncoated samples. We found no difference between the spray containing whey protein isolate alone and the spray containing whey protein isolate and an antioxidant.

Table 2. Average Weight of Coatings Applied to Fish Samples

Treatment	Weight of First Component (g)	Weight of Second Component (g)
None uncoated	NA	NA
10% whey protein isolate	1.613 ± 0.459	NA
10% whey protein isolate/antioxidant overspray	1.267 ± 0.200	0.758 ± 0.174
Emulsion of 10% whey protein isolate and 10% Myvacet 9-08	3.213 ± 0.483	NA
Emulsion of 10% whey protein isolate and 10% Myvacet 9-45	2.453 ± 0.257	NA
10% whey protein isolate/Myvacet 9-08 overspray	1.270 ± 0.179	0.584 ± 0.116
Powder of whey protein isolate/Myvacet 9-08 overspray	0.469 ± 0.067	0.988 ± 0.220
Powder of whey protein isolate /Myvacet 9-45 overspray	0.453 ± 0.055	0.413 ± 0.208
Myvacet 9-08 overspray only	0.995 ± 0.310	NA
Myvacet 9-45 overspray only	1.780 ± 0.209	NA

Note: Average weight of the fish samples to which coating was applied was 48 gr. NA = not applicable.

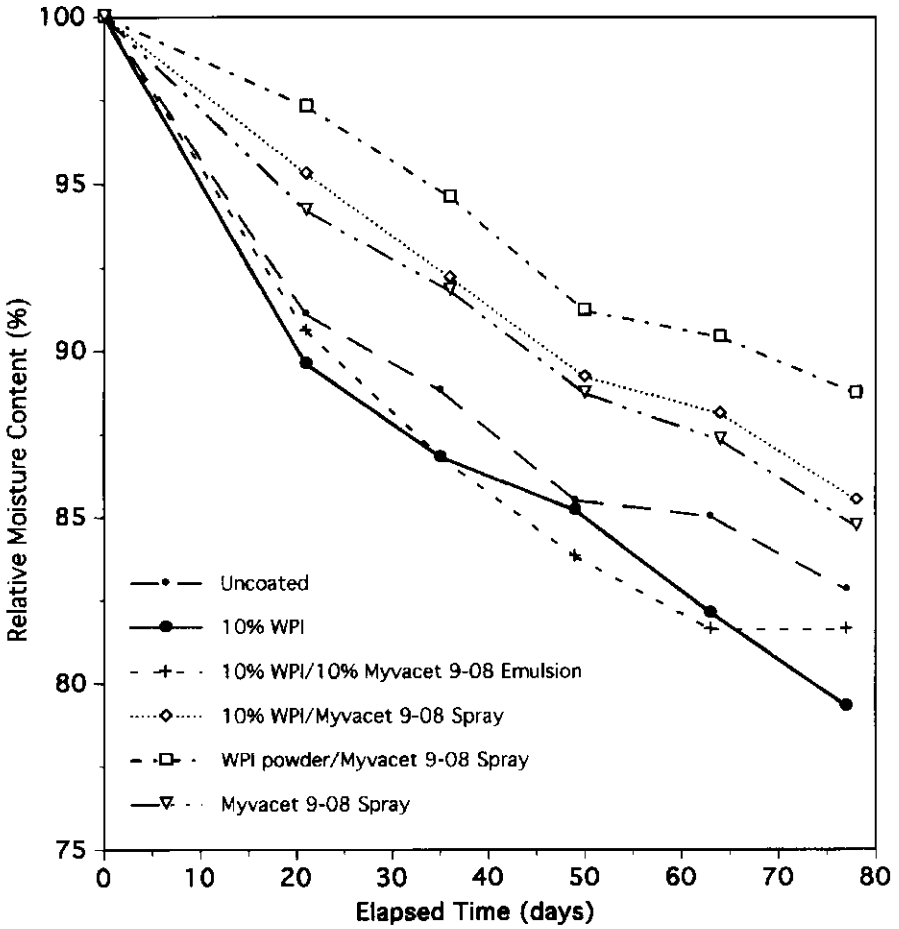

Figure 1. Relative moisture content of pieces of king salmon coated with various edible films and stored at –23°C. WPI = whey protein isolate.

Samples coated with a spray of acetylated monoglyceride had less moisture loss than uncoated samples. During the first 3 weeks, the rate of moisture loss from samples coated with a powder of whey protein isolate followed by a spray of acetylated monoglyceride was 0.007 g/cm^2 per week. This compares with losses of 0.019 g/cm^2 per week from uncoated samples and 0.069 g/cm^2 per week from pure ice. In the uncoated samples, the rate of moisture loss slowed to 0.007 g/cm^2 per week during the next 8 weeks, indicating that the desiccated surface of the uncoated fish was itself a barrier to moisture loss. During the same 8-week period, the average rate of loss from the coated samples was 0.008 g/cm^2 per week.

Peroxide values did not show any significant differences until the fifth week of testing. Figure 2 gives the peroxide values for the different treatments at 5 weeks. All the coatings reduced the peroxide value significantly compared with the uncoated samples. Pieces coated with a powder of whey protein isolate followed by a spray of Myvacet 9-08 had the lowest peroxide value. The order of effectiveness of the other coatings was (1) emulsion containing whey protein isolate and Myvacet, (2) spray of whey protein isolate followed by a spray of antioxidant, (3) spray of Myvacet only, (4) spray of whey protein isolate followed by a spray of Myvacet. The peroxide value was highest for pieces coated with a spray of whey protein isolate alone (Figure 2). At the sampling times of 7, 9, and 11 weeks, differences in the peroxide values between uncoated samples and the coated samples were not as apparent, but all coated samples had lower values than the uncoated controls (data not shown).

The peroxide value is a measure of the hydroperoxide content of the sample. Hydroperoxides are initial products of lipid oxidation, which then react further to form secondary products. The rate of formation of hydroperoxide greatly exceeds the rate of decomposition during the early stages of lipid oxidation and correlates reasonably with oxidation in fish oils (Hardy, 1979).

The rate of lipid oxidation in fatty fish such as salmon is affected by a number of factors. The lipid content and composition of the fish flesh can vary greatly, depending on the age, maturity, and size of the fish and the position in the fish where the sample is taken (e.g., head to tail, back or belly flap, dark or light meat) (Ackman, 1979; Stansby and Olcott, 1963). Fish such as salmon contain a wide variety of types of lipids, some very unsaturated and highly reactive. The disposition of lipid within the tissue also affects the rate of oxidation; dark muscled flesh tends to oxidize more rapidly than light flesh (Hardy, 1979). Nonlipid components, such as metals and tocopherols, can also affect the rate of lipid oxidation (Hardy, 1979). These factors contribute to the complexity of the data we obtained. Nonetheless, the reduction in peroxide values achieved with the coatings tested clearly indicated a significant reduction in lipid oxidation for salmon.

Summary and Conclusions

Edible films were formed from casein, whey protein, and soy protein, either alone or in combination with acetylated monoglycerides or other lipids. The addition of lipids to protein films in an emulsion system reduced permeability of the films to moisture. Casein and whey protein films were also excellent oxygen barriers. Model gels were developed and used to test edible film coatings under freezing conditions. Edible film coatings consisting of acetylated monoglycerides alone

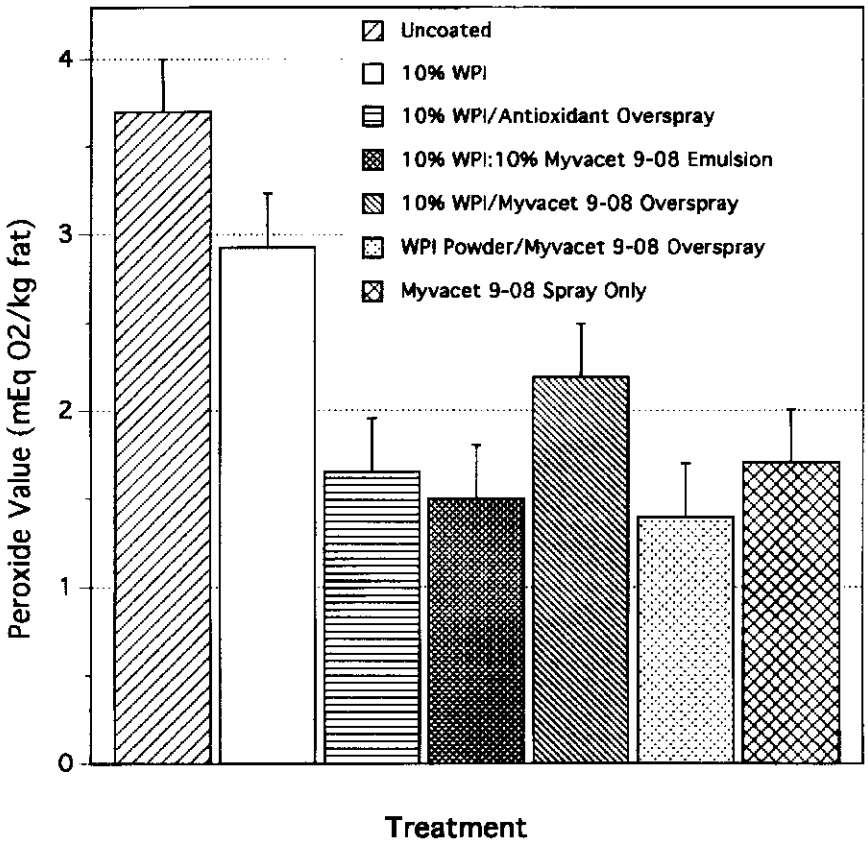

Figure 2. Peroxide value at 5 weeks of pieces of king salmon coated with various edible films and stored at –23°C. WPI = whey protein isolate.

or sprayed over protein coatings reduced moisture loss when applied to frozen salmon. Edible film coatings containing antioxidants or acetylated monoglycerides reduced the peroxide value of the samples and slowed the oxidation of the fish lipids, thus extending the storage life of frozen fish.

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The Importance of Transport Processes in Recruitment of Rockfishes (Genus *Sebastes)* to Nearshore Areas of Monterey Bay, California

Moss Landing Marine Laboratories
R/F-142
1991–94

Valerie Loeb, Mary Yoklavich, and Gregor Cailliet

Rockfishes (*Sebastes* spp.) support important and heavily exploited sport and commercial fisheries in central California; their larvae dominate inshore ichthyoplankton assemblages (Loeb et al., 1983 a, 1983b; MacGregor, 1986; Wallace, 1988; Moser et al., 1993). Factors affecting the survival of young fishes, including hydrography, climate, densities of prey and predator, and spawning seasonality, are critical determinants of recruitment. This 2-year project was based on the hypothesis that the processes of physical transport affect the distribution of young rockfishes, thereby influencing the strength and timing of recruitment to nearshore areas along the central California coast. The arrival of pelagic juvenile rockfish to subtidal areas of Monterey Bay appears to be associated with upwelling events (Ven Tresca et al., 1990). The recurring plumes of cold water from a distinct upwelling center near Davenport (Tracy, 1990; Schwing et al., 1991; Rosenfeld et al., 1994), a relatively unfished area to the north of Monterey Bay, may be important vehicles of transportation for young rockfishes into Monterey Bay. Similarly, northward flow of water upwelled off Pt. Sur may transport young rockfishes into the Bay from the south.

The objectives for the 1991–1993 field seasons were to assess (1) distribution and potential transport of rockfish larvae from nearshore source areas before the upwelling season and (2) the role of upwelling-related processes in advecting pelagic juvenile rockfishes into Monterey Bay.

Accomplishments

The 1991–93 study period off central California was characterized by a prolonged El Niño event. Anomalously warm, low–salinity water in nearshore areas during much of our study suggested an onshore displacement of the California Current. Upwelling was reduced and delayed relative to that of other years, and distinct persistent upwelling plumes were not evident within the survey area during the sampling periods. However, the 2 years differed in the intensity, duration, frequency and direction of wind events that can affect transport.

During early spring 1992, wind speed was somewhat higher than during the same period in 1991 (Figure 1), but wind direction reversed during February and March 1992, blowing predominantly northward for 2–12 days at a time (Figure 2A). Coincidentally, water temperatures in the upper 60 m were warmest during wind reversals (Figure 2A), and increased to 15°C by April 2. Wind reversals associated with warm and less saline water indicate onshore advection of surface water. The conductivity-temperature-depth (CTD) vertical temperature profiles during the spring surveys of ichthyoplankton off Davenport, California, in 1992 indicated an increasingly stratified and warm water column (Figure 3). During May and June 1992, the alongshore winds were generally southward and more typical for this time of year (Figure 1). However, wind speed was low relative to that of the previous year, persistent upwelling was not evident in Advanced Very High Resolution Radiometer (AVHRR) satellite images of sea surface temperature, and calm events were frequent. Cool-water plumes occurred along the coast to the north and south of the Bay in July, which were the first signs of upwelling at the surface (Figure 4).

Winds during early spring 1993 were variable, but unlike those in 1992 they were mostly to the south and more typical of this season (Figure 1). Persistent southward-blowing wind commencing in early March 1993 resulted in cooler (10–14°C) temperatures in the upper 80 m of the water column off Davenport by early April (Figure 2B). This was the first evidence of upwelling during either year of the ichthyoplankton surveys, as indicated by the 10.0–10.5°C isotherm sloping up toward shore in the 6 April 1993 CTD vertical profile (Figure 3). Wind direction reversed in mid-May 1993 (Figure 1), again suggesting a brief period of potential onshore advection. In June and July intense winds favorable to upwelling resulted in cold water at the surface to the north and south of the Bay; however, upwelling plumes did not persist during the juvenile rockfish surveys. During May and June, a warm meander developed off Monterey Bay (Figure 5), possibly indicating flow of water toward the equator on the eastern (shoreward) edge (Rosenfeld et al., 1994).

Extended El Niño conditions and absence of persistent upwelling plumes during our study directly affected implementation of the project (e.g., sampling design and schedules for surveys of juvenile rockfish) and results during both years. Despite the absence of distinct thermal fronts, differences in wind regimes and related water transport, especially during early spring surveys of ichthyoplankton, likely influenced rockfish recruitment in the 2 years. We evaluated between-year differences in the distribution, abundance, growth, and species and size composition of young rockfish, and interpreted these in terms of recruitment success and potential source areas.

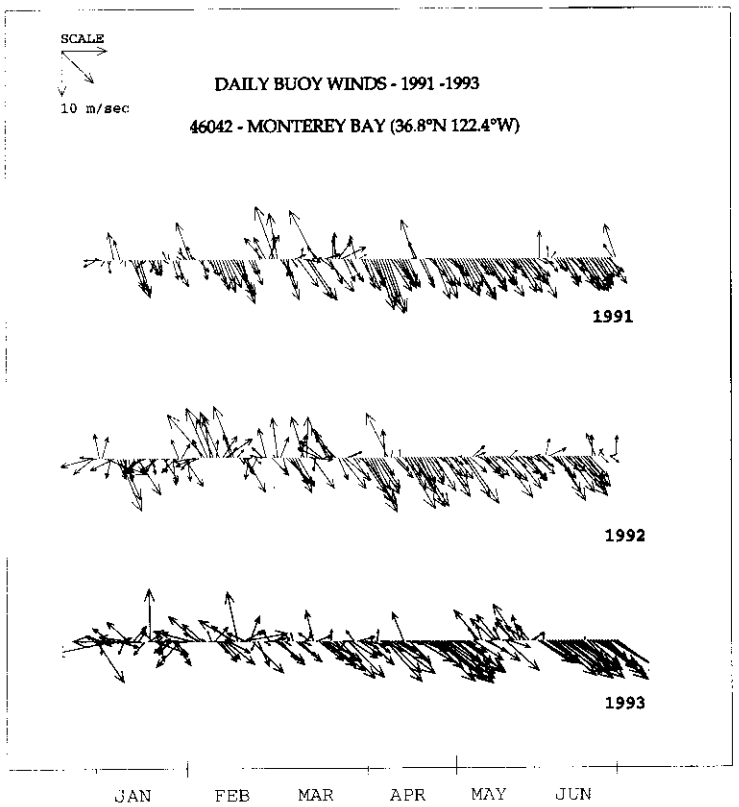

Figure 1. Daily-averaged wind vectors measured at buoy 46042 in Monterey Bay (north is to top of page) during 1991–1993.

Surveys of Larval Rockfish

Surveys of ichthyoplankton were conducted aboard Moss Landing Marine Laboratories research vessel *Ricketts* during daytime at five stations along a northeast-southwest transect over depths of 15, 30, 60, 100, and 200-m off Davenport, California (Figure 6). These stations were located 1, 3, 7, 13, and 19 km from shore. During 1991–92, samples were collected at approximately 2 week intervals from early December to April; less frequent sampling extended through June because of the absence of expected upwelling events during the spring. Sampling between January and April 1993 was limited by frequent storms and rough seas (Table 1).

Samples of larvae were collected with a 70-cm (mouth diameter) bongo net without a closing mechanism. Both nets were made of 0.505-mm mesh black Nitex. The volume of water filtered per sample (30–465 m^3) was estimated by using calibrated mechanical flowmeters positioned in the mouth of each net. Tows were made parallel to bathymetry at 1–3 kt for 2–20 min (depending on depth) and obliquely from near bottom to surface at nearshore stations and from 200 m at the offshore station. Three replicate tows were done at most stations . Oceanographic parameters (e.g., conductivity, temperature, and pressure) were measured from near bottom to the surface at each station with a calibrated CTD profiler.

Plankton samples were preserved at sea, one sample from each bongo pair in 5% formalin and the other in 80% ethanol. Rockfish larvae were sorted under dissecting microscopes from each paired sample for all replicates per station. Formalin-preserved rockfish from all replicates were counted for estimates of abundance. Only one ethanol-preserved replicate per station was processed for species identification. Standard length (or notochord length for preflexion larvae) was measured to the nearest 0.1 mm for shortbelly rockfish (*S. jordani*) and blue rockfish (*S. mystinus*) preserved in ethanol. Otoliths were removed for age determination from a subsample of the dominant species (shortbelly rockfish); the methods of Yoklavich and Bailey (1990) were used to determine larval age. Hatch dates were calculated by using a linear growth model to estimate age from lengths of all larval shortbelly rockfish and subtracting age from date of capture.

Seven rockfish species were identified: shortbelly, blue, bocaccio (*S. paucispinis*), greenspotted (*S. chlorostictus*), squarespot (*S. hopkinsi*), stripetail (*S. saxicola*), and cowcod (*S. levis*; Table 2). Two groups of species were identified on the basis of shared pigment patterns: The "copper complex" group includes copper (*S. caurinus*), gopher (*S. carnatus*), black-and-yellow (*S. chrysomelas*), kelp (*S. atrovirens*), china (*S. nebulosus*), quillback (*S. maliger*), and brown (*S. auriculatus*) rockfish. The *Sebastosomus* group includes black (*S. melanops*), olive (*S. serranoides*), yellowtail (*S. flavidus*), widow (*S. entomelas*), and bank (*S. rufus*) rockfish. Identification of larval blue and copper complex rockfish was aided by reference to specimens reared in the laboratory during a previously funded Sea Grant research project (Wold, 1991; Moreno, 1993).

Larval abundance was compared among the five stations along the onshore-offshore transect. Abundance was calculated for each replicate at each station by multiplying the number of larvae by the depth of tow and dividing by the volume of water filtered per sample. Abundance was scaled by multiplying by 10 (i.e., number of larvae per 10 m^2).

The 130 bongo net tows made during 10 surveys in 1991–1992 contained 9389 rockfish larvae. Rockfish larvae were relatively abundant from January to April 1992, with the largest catches occurring in early February at all but one station (Figure 7A; Table 2). Abundance consistently was highest at the three offshore stations (i.e., 7, 13, 19 km). While abundance declined steadily from

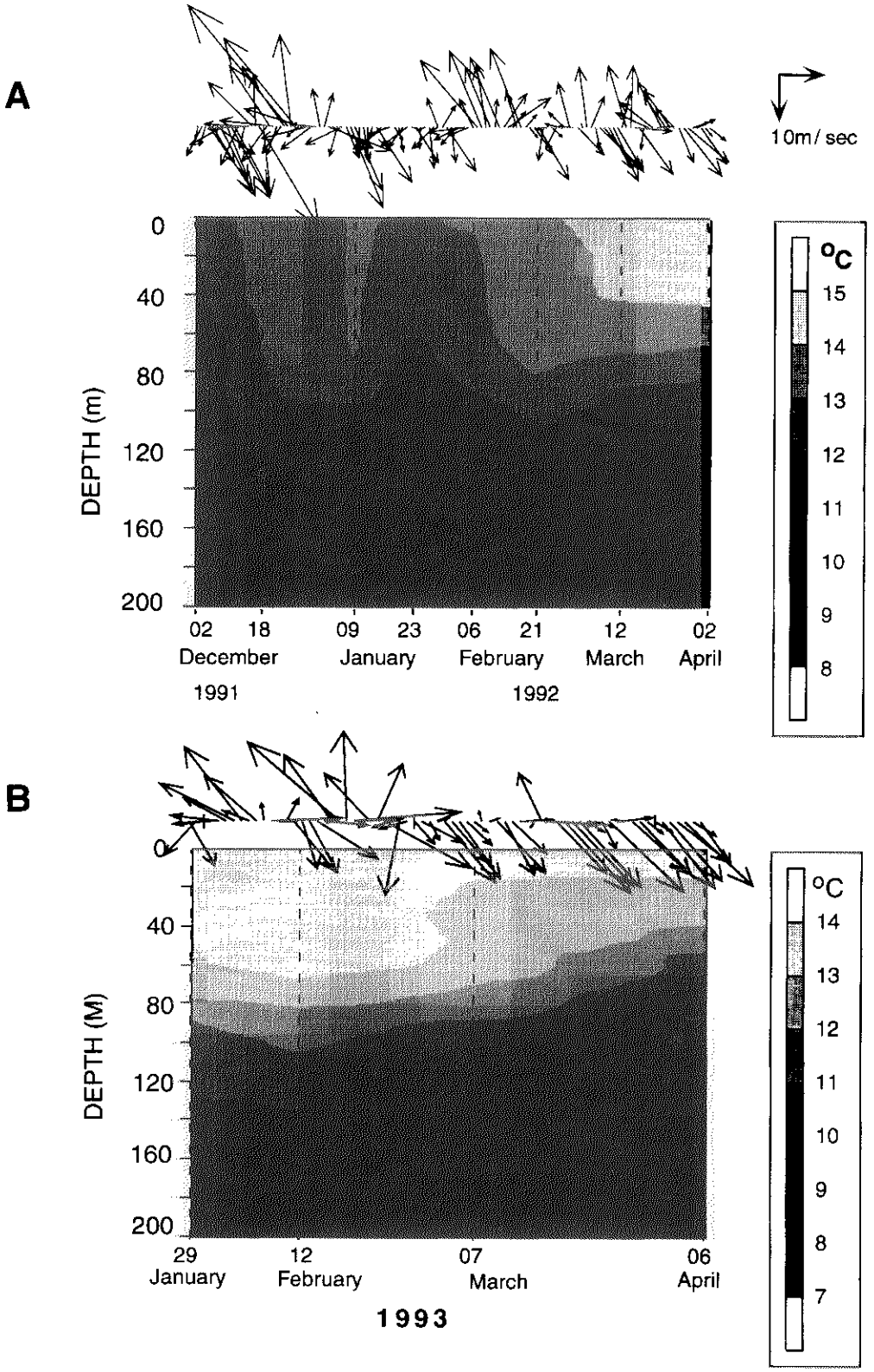

Figure 2. Daily-averaged wind vectors measured at buoy 46042 in Monterey Bay (north is to top of page) and temperature variation with time in upper 200 m at station (19 km) during ichthyoplankton surveys off Davenport, California. A, 2 December 1991–2 April 1992. B, 29 January–6 April 1993. CTD sampling dates are indicated with dashes.

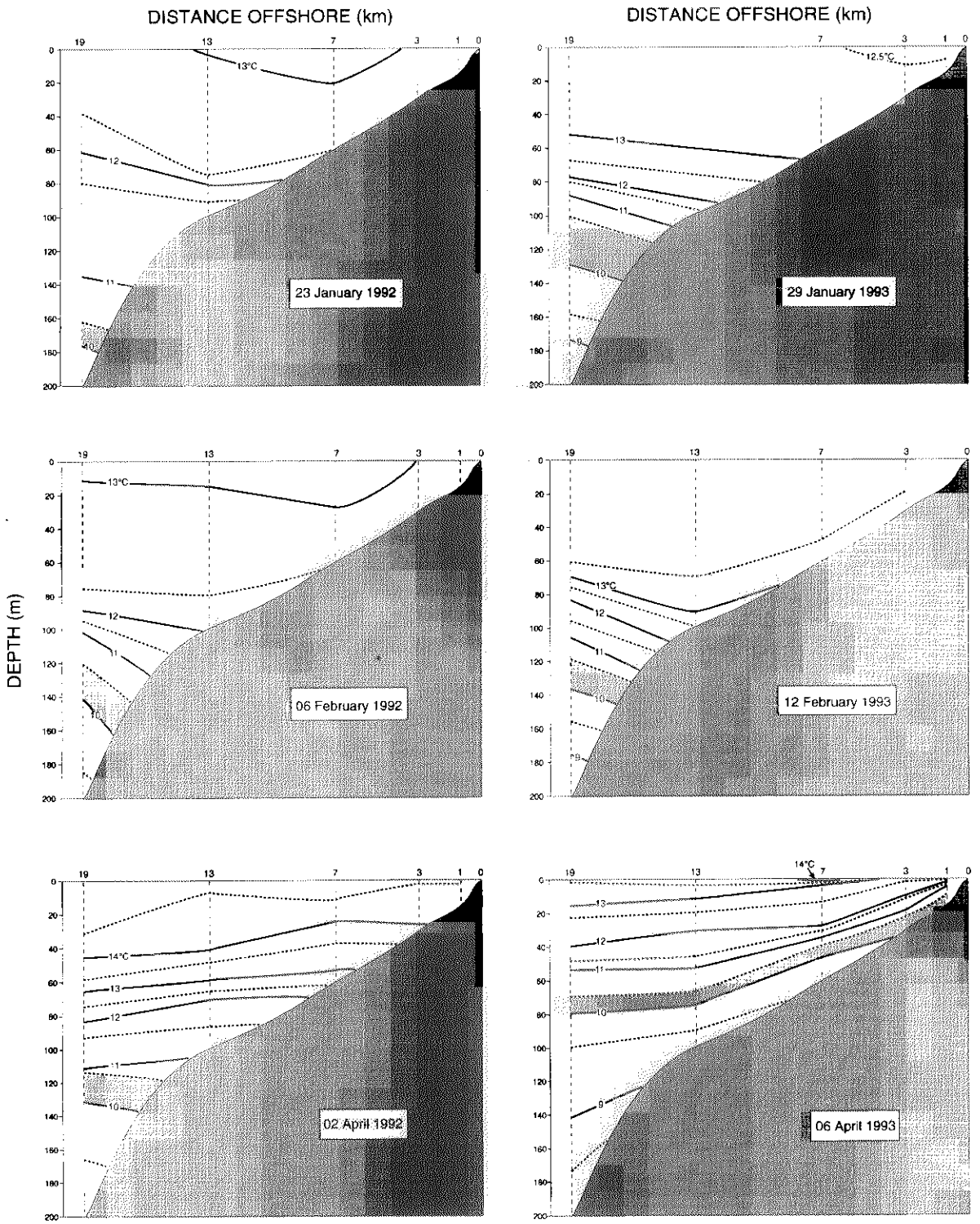

Figure 3. Vertical temperature profiles along a sampling transect off Davenport, California, during January, February, and April in 1992 and 1993. The five CTD stations are indicated by distance offshore. The 10.0–10.5 °C isotherm is shaded for comparative purposes.

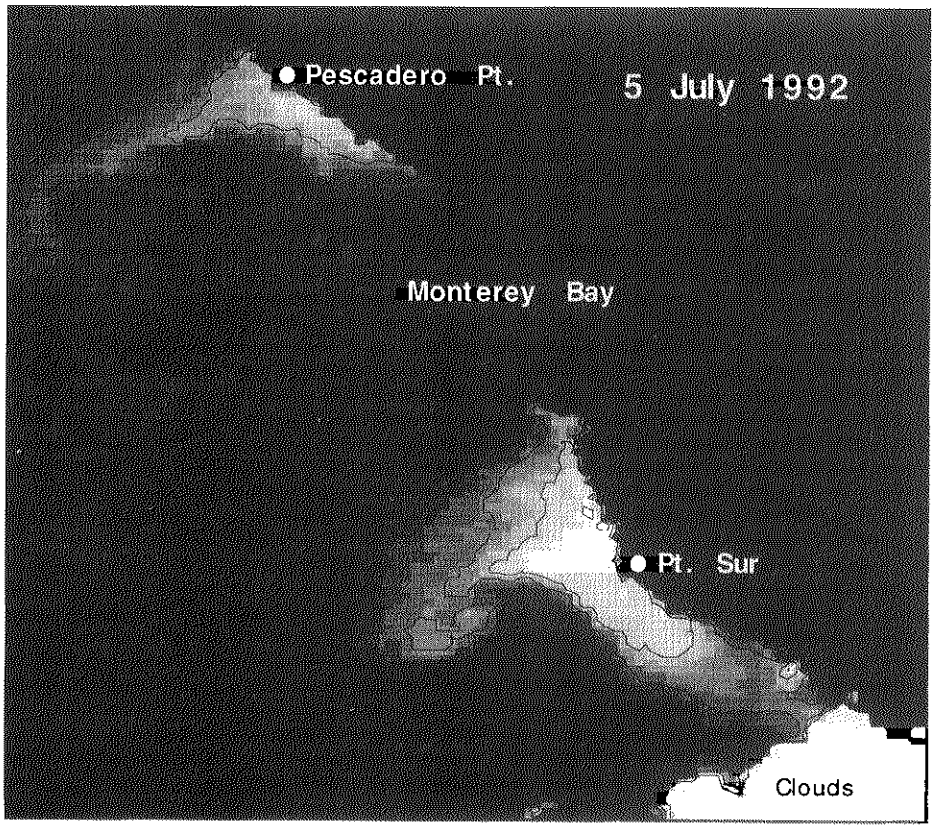

Figure 4. AVHRR satellite image of sea surface temperatures off central California coast 5 July 1992, indicating upwelling plumes have developed off Pescadero Point (north) and Point Sur (south). Cold water is represented by lightest shades.

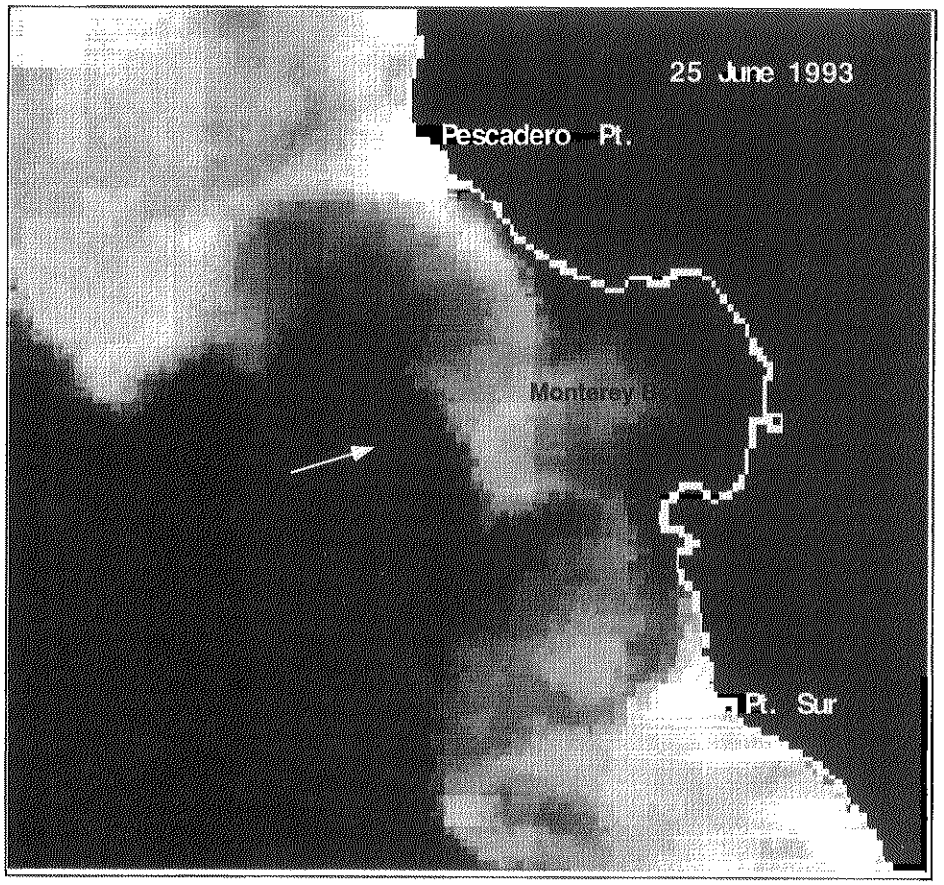

Figure 5. AVHRR satellite image of sea surface temperature off Monterey Bay. Warmest temperature is represented by darkest shade. Relatively cool water occurs of Pescadero Point and Point Sur; arrow indicates a warm meander in the California Current.

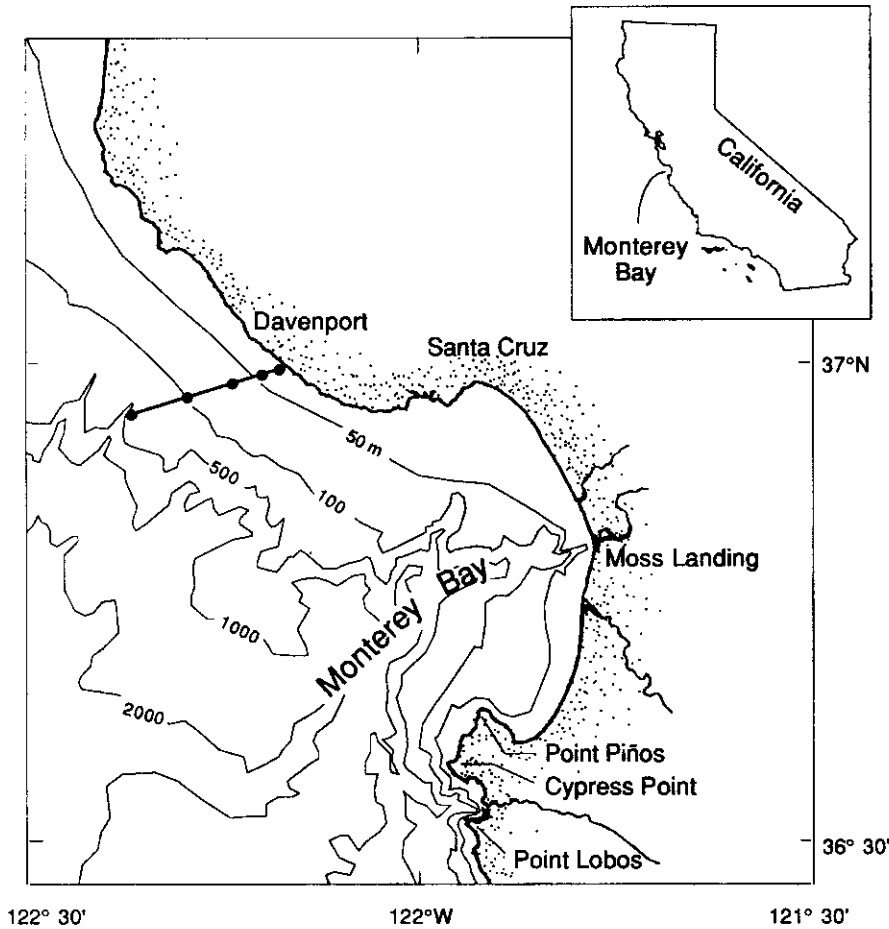

Figure 6. Monterey Bay study area and larval fish sampling transect off Davenport, California.

Table 1. Dates of Collection and Number of Replicates Taken Along an Onshore-Offshore Transect off Davenport, California During Winter–Spring Months 1991–1993*

Date	15 m (1 km)	30 m (3 km)	60 m (7 km)	100 m (13 km)	200 m (19 km)
1991–1992					
02 December	3	3	2	3	3
18 December	3	3	3	3	3
09 January	3	3	3	3	3
23 January	3	3	3	3	3
06 February	3	3	3	3	3
21 February	3	3	3	3	3
12 March	3	3	3	3	3
02 April	3	3	3	3	3
04 May	–	–	–	1	1
16 June	–	3	3	3	–
Subtotal	24	27	26	28	25
1993					
29 January	3	3	3	3	3
12 February	–	–	3	3	3
07 March	–	3	–	–	3
06 April	–	3		3	3
Subtotal	3	9	6	9	12
Total	27	36	32	37	37

*Stations are defined by bottom depth (m) and distance from shore (km).
(– indicates no samples were taken.)

Table 2. Abundance (Number per 10 m^2) of Larval Rockfishes in Bongo Net Tows Collected off Davenport, California, December–June 1991–1992 and January–April 1993

1991–1992	2 Dec					18 Dec					9 Jan					23 Jan					6 Feb				
	1	3	7	13	19	1	3	7	13	19	1	3	7	13	19	1	3	7	13	19	1	3	7	13	19
S. jordani	–	–	–	–	–	–	–	–	4	8	–	–	34	29	186	4	–	144	269	21	–	–	152	656	166
S. mystinus	–	–	–	–	–	–	3	–	–	–	–	7	15	–	38	2	4	85	21	109	–	–	109	180	90
S. hopkinsi(?)	–	–	–	–	–	–	3	12	12	–	–	–	49	4	34	–	–	25	25	11	–	–	67	46	54
S. saxicola	–	–	–	–	–	–	–	–	–	4	–	–	4	–	17	–	–	11	17	189	–	–	4	8	54
Copper complex	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–	7	–	4	3	4	38	17	27
S. paucispinnis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	21	64	32	–	–	4	8	18
Sebastosomus	–	–	–	–	–	–	–	8	–	–	–	–	4	–	–	–	–	–	–	4	–	–	42	–	36
S. chlorostictus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. levis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	4	–
Unidentified	–	3	–	13	37	4	8	19	56	62	–	3	42	69	169	8	4	113	191	186	3	61	160	3127	714
Damaged	–	–	–	3	–	–	–	4	–	–	–	–	4	–	47	–	–	18	7	21	–	4	29	75	81
Total Standardized Abundance	–	3	–	16	37	4	14	47	71	74	–	10	151	101	512	14	8	423	593	575	6	69	610	4122	1239

1992 (cont.)	21 Feb					12 Mar					2 Apr					4 May		16 June			Total Standardized Abundance	Total Abundance (%)
	1	3	7	13	19	1	3	7	13	19	1	3	7	13	19	13	19	3	7	13		
S. jordani	–	–	43	131	–	–	4	41	380	823	–	–	–	–	372	49	3	–	–	–	3520	29
S. mystinus	–	–	9	26	22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	719	6
S. hopkinsi (?)	–	–	43	33	18	–	–	4	23	14	–	–	–	–	4	4	3	–	–	–	487	4
S. saxicola	–	4	–	23	4	–	–	–	14	29	–	–	–	19	–	–	–	–	–	–	401	3
Copper complex	–	–	17	3	4	8	4	8	–	–	6	7	4	27	–	–	–	–	10	4	207	2
S. paucispinnis	–	–	–	3	13	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	189	2
Sebastosomus	–	–	–	3	4	–	–	–	–	–	–	–	–	–	4	–	–	–	–	4	109	1
S. chlorostictus	–	–	–	–	–	–	–	–	–	–	–	–	–	15	–	–	–	–	–	4	19	0.2
S. levis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	12	0.1
Unidentified	–	12	72	311	88	–	–	4	135	91	–	–	4	8	29	185	128	–	–	–	6118	50
Damaged	–	–	26	7	9	–	–	4	28	29	–	–	–	–	–	–	–	–	–	–	394	3
Total Standardized Abundance	–	16	208	540	162	8	8	62	585	987	6	7	7	68	410	238	138	–	10	12	12175	100

1993	29 Jan					12 Feb			7 Mar		6 Apr			Total Standardized Abundance	Total Abundance (%)
	1	3	7	13	19	7	13	19	3	19	3	13	19		
S. jordani	–	–	21	153	189	2	1274	92	–	274	–	44	199	2249	46
S. saxicola	–	–	–	32	76	–	91	70	–	9	–	–	4	281	6
S. mystinus	–	–	5	90	167	–	25	15	–	–	–	–	–	303	6
S. hopkinsi (?)	–	–	11	11	–	–	147	44	11	–	–	–	–	223	5
Sebastosomus	–	–	–	48	11	–	45	19	–	7	–	–	–	130	3
Copper complex	–	–	5	–	–	–	35	15	–	2	–	–	–	57	1
S. paucispinnis	–	–	–	11	5	–	–	11	–	–	–	–	4	31	1
S. chlorostictus	–	–	–	–	–	2	5	–	–	–	–	–	–	7	0.1
S. levis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Unidentified	–	8	53	99	156	23	390	282	–	126	–	39	188	1364	28
Damaged	–	–	11	37	–	–	243	–	–	–	–	–	–	291	6
Total Standardized Abundance	–	8	106	480	604	28	2255	549	11	418	–	83	393	4937	100

Note: Dashes = 0 larvae collected.

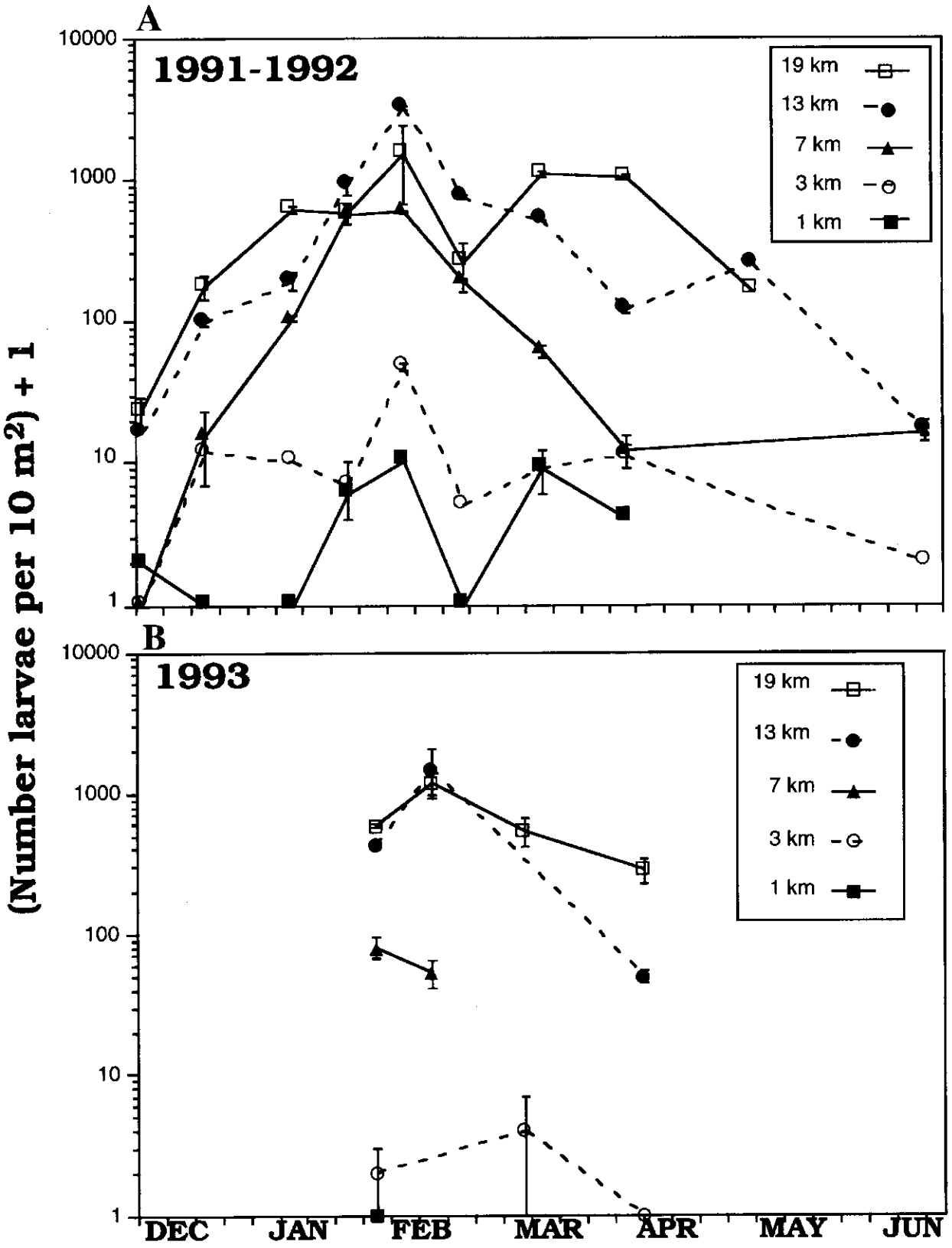

Figure 7. Larval rockfish abundance at five stations along an onshore-offshore transect, Davenport, California. Each point is the average of one to three replicates; error bars = standard error. A, 2 December –16 June 1992. B, 29 January–6 April 1993.

early February through April at the 7- and 13-km stations, numbers of larvae remained relatively high at the station farthest from shore (19 km).

Four surveys conducted in 1993 included 39 bongo net tows containing 3492 rockfish larvae. Similar to findings in collections made in 1992, the highest abundance occurred in February (Figure 7B; Table 2). In contrast to the previous year, high catches of rockfish larvae occurred only at the two offshore stations. Abundances averaged over January through April surveys were similar between years (343 larvae/10 m^2 in 1992; 381 larvae /10 m^2 in 1993), but distribution patterns differed. Significantly greater numbers of larvae occurred at the onshore stations (1, 3, and 7 km) during January and February 1992 than in the same months in 1993. This nearshore concentration of larvae early in 1992 is possibly the result of wind-related onshore transport of surface water, as discussed in the preceding text.

We were able to identify the species or group of species of 47% and 66% of the rockfish larvae collected in 1992 and 1993, respectively (Table 2). Shortbelly rockfish were the dominant species both years and accounted for a larger percentage of total catch in 1993 (46% vs. 29%). Shortbelly larvae occurred from late December to early May, largely in the offshore samples (13 and 19 km). On the basis of larval ages and collection dates, we concluded that hatching of shortbelly rockfish extended from December to May, with a median hatch date in mid-February both years (Figure 8). Growth of this species did not differ among months or between years. In the 1992 samples, larval shortbelly rockfish were significantly larger at onshore than at offshore stations. This coincides with indications of onshore advection of water (i.e., reversals in wind direction, warm and less saline water), suggesting retention of larvae nearshore in 1992. Initiation of upwelling in March and April of 1993 and fewer larvae at onshore stations suggest greater offshore transport during these months.

Although much less numerous than shortbelly rockfish, blue, stripetail, and squarespot rockfish larvae also were abundant (4–6% of the total); the last two species were relatively more abundant in 1993 (Table 2). Interestingly, squarespot rockfish are a southern California species, rarely found north of Monterey Bay, but they were a dominant species in our larval and juvenile surveys and abundant in samples collected near deep rock outcrops in the Bay in the fall of 1992–1993 (M. Yoklavich, unpublished data). The copper complex group, although representing only 1–2% of the total catch, generally could be found in the nearshore samples.

Surveys of Pelagic Juvenile Rockfish

We collected pelagic juvenile rockfishes with a 24-m midwater trawl deployed from the 15-m fishing vessel *Good News*, which was chartered by the California Department of Fish and Game (CDFG) for 12 days in 1992 and 16 days in 1993. Fishing depth ranged from 26 m at shallow stations to 77 m; fishing time was approximately 15 min. Sampling operations were flexible and were based on prevailing hydrographic conditions monitored in the field and from near real-time AVHRR satellite images of sea surface temperature. Because of this flexibility, we extended our study period and sampled relatively large aggregations of pelagic juvenile rockfishes occurring late in the season both years.

Transect stations were located at thermal discontinuities indicated by underway measurements of sea surface temperature or at evenly spaced intervals off Davenport, northern Monterey Bay, southern Monterey Bay, Point Piños, Cypress Point, and Point Lobos (Figure 6). Between two and six trawls were made each night, and each transect generally included four to five stations. A CTD cast was made at each station. Rockfishes were rough sorted onboard and frozen. After final identification and standard measurements of length, rockfish were transferred to ethanol.

In 1992 sampling was conducted during early May, June, and July (Table 3). AVHRR satellite images obtained late in April indicated uniformly warm sea surface temperatures in coastal waters, and warm, low-salinity waters characterized the upper water column (0–30 m) in the Monterey Bay area during survey 1 (May 4–9, Table 3). Twenty tows yielded only one juvenile rockfish (Figure 9A). Although a satellite image obtained on May 23 indicated some coastal upwelling off Pescadero Point (north of Davenport) and Point Sur (south of Cypress Point), it was not apparent in subsurface temperature and salinity profiles off Davenport or Monterey during survey 2 (June 2–6). In fact, relatively warm, low-salinity water prevailed in the southern part of the bay at this time (Table 3). A total of 174 pelagic juvenile rockfishes were collected in 20 tows during survey 2. The majority of these (158) were collected at the southern transect stations off Cypress Point and Point Piños (Figure 9B). During survey 3 (July 7–10), decreased temperature and increased salinity in the upper water column reflected the influence of upwelling off Pescadero Point and Point Sur (Figure 4). This was most apparent in the southern part of the bay where the average temperature of the upper water column was 1.8°C colder than the temperature in the previous month (Table 2). Sixteen tows collected 58 pelagic juvenile rockfishes during survey 3; nearly equal numbers were found in the northern and southern parts (Table 3; Figure 9C).

Twelve rockfish taxa were represented in the midwater trawl samples in 1992 (Table 4), including 10 species, the copper complex, and the *Sebastomus* group. Shortbelly rockfishes were most abundant and accounted for 131 (56%) of the 233 fish collected. Members of the copper complex also were relatively abundant (18%).

The size of the juvenile rockfish in 1992 was generally 10–30 mm (Figures 10A and 10B); average length for most species during each survey was less than 25 mm. These fish were small compared with juveniles collected in previous years

Table 3. Pelagic Juvenile Rockfish Collected During Midwater Trawl Surveys in the North and South Monterey Bay Areas in 1992 and 1993

	1992								
	Survey 1 4–9 May			Survey 2 2–6 June			Survey 3 7–10 July		
	North	South	Total	North	South	Total	North	South	Total
No. Tows	10	10	20	10	10	20	6	10	16
No. Fish	1	0	1	16	158	174	28	30	58
No. Fish/Tow	0.1	0.0	0.1	1.6	15.8	8.7	4.7	3.0	3.6
MeanT	13.1	13.1		13.1	13.8		12.6	12.0	
Std T	0.7	0.4		0.4	0.3		0.6	0.5	
Mean S	33.30	33.21		33.38	33.20		33.34	33.38	
StdS	0.13	0.06		0.08	0.07		0.15	0.11	

	1993								
	Survey 4 5–11 May			Survey 5 25 May–6 June			Survey 6 20 June–9 July		
	North	South	Total	North	South	Total	North	South	Total
No. Tows	10	–	10	–	20	20	20	12	32
No. Fish	37	–	37	–	988	988	515	4825	5340
No. Fish/Tow	3.7	–	3.7	–	49.4	49.4	25.8	402.1	166.9
No. Fish*		–		–				259	774
No Fish/Tow*		–		–				21.6	24.2
MeanT	10.3	–		–	13.4		11.2	12.3	
Std T	0.4	–		–	0.6		0.5	0.3	
Mean S	33.48	–		–	33.12		33.5	33.44	
StdS	0.12	–		–	0.13		0.13	0.11	

Note: Mean and standard deviations of temperature (T °C) and salinity (S) are derived from integrated 0- to 30-m CTD values.
*Excludes three large, probably near-bottom catches made during 1993. (– indicates no samples were taken.)

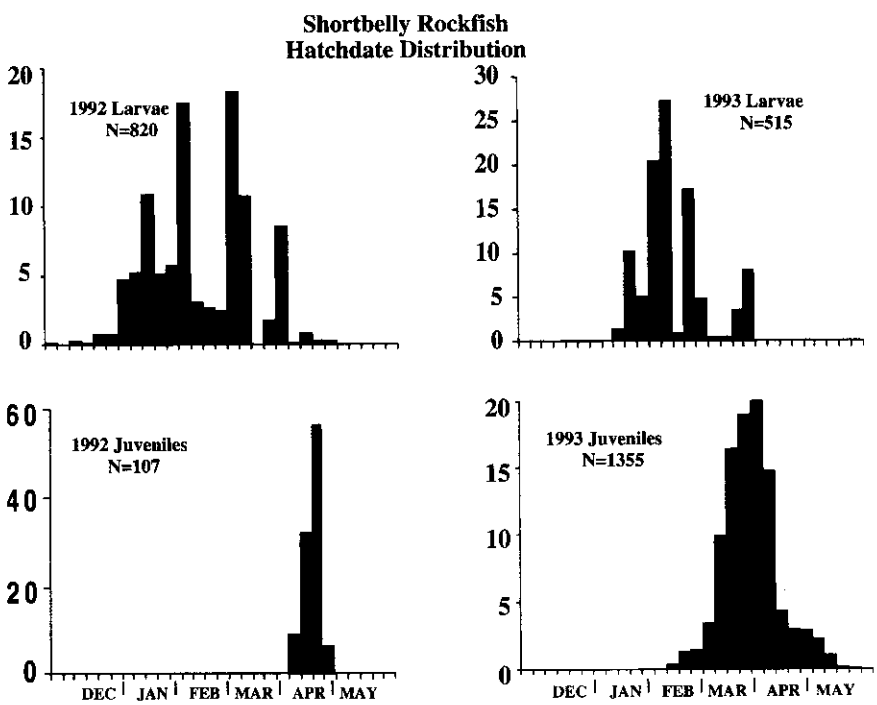

Figure 8. Hatch date distributions for shortbelly rockfish, estimated from age of larvae collected from January through April in 1992 and 1993 and of juveniles collected in June 1992 and May–July 1993.

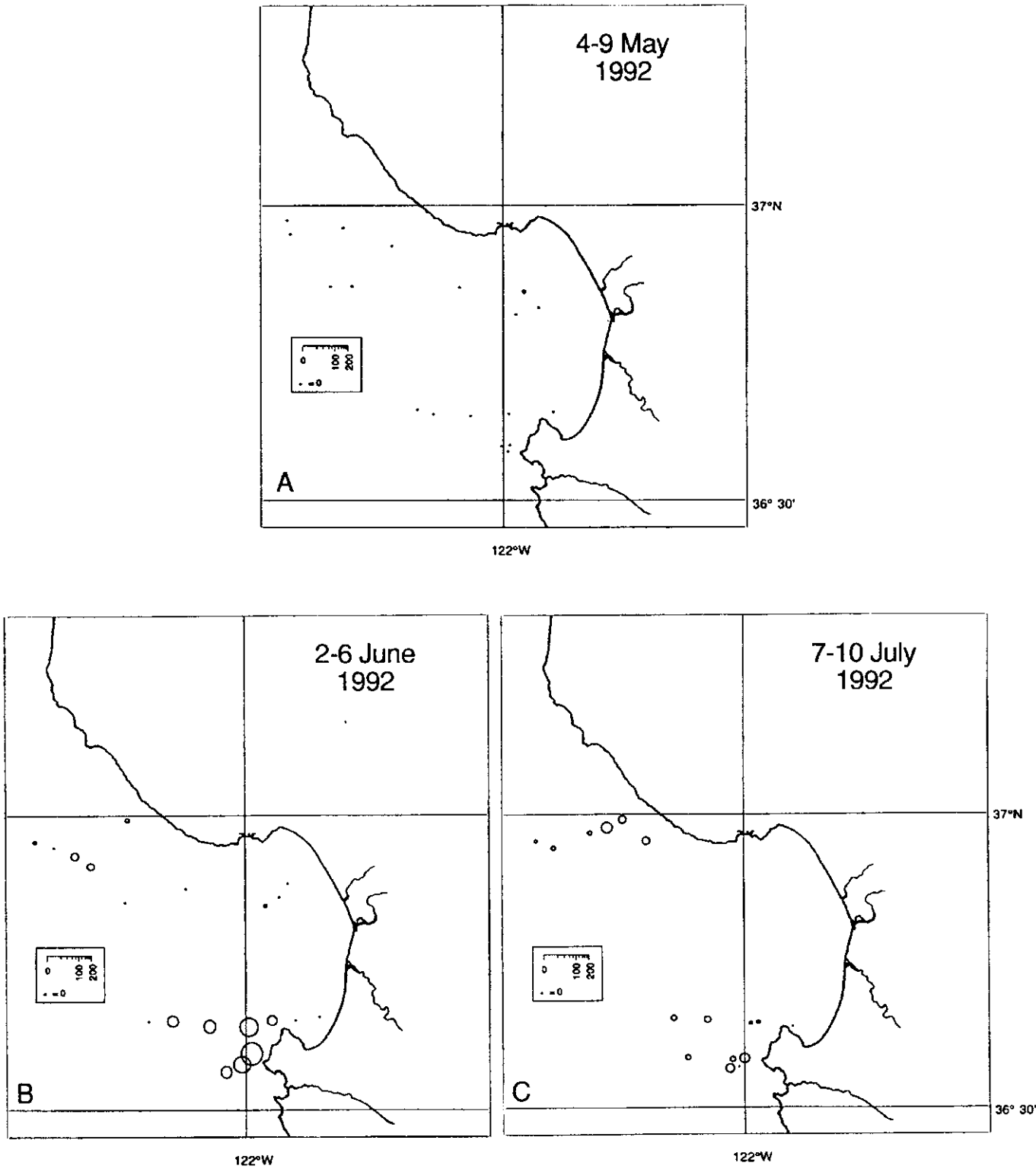

Figure 9. Location of net tows and abundance of pelagic juvenile rockfishes collected in 1992. A, Survey 1 (4–9 May). B, Survey 2 (2–6 June. C, Survey 3 (7–10 July).

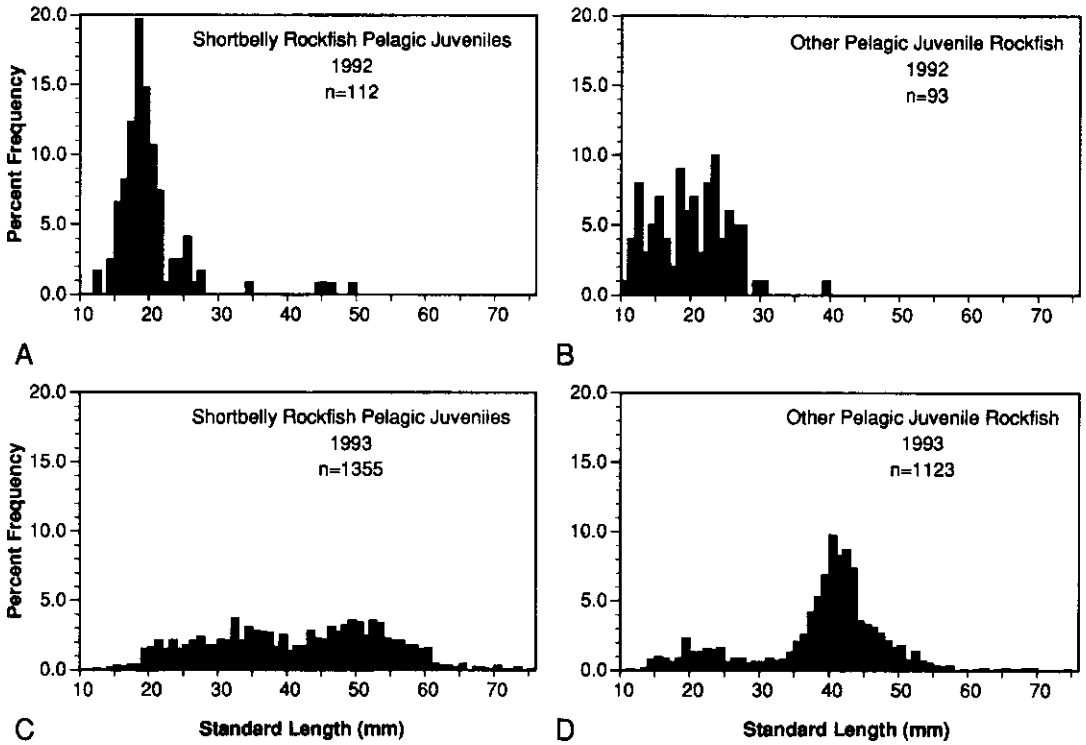

Figure 10. Length frequency distributions for shortbelly rockfish juveniles (A) and other juvenile rockfishes (B) collected in 1992 and shortbelly rockfish juveniles (C) and other juvenile rockfishes (D) collected in 1993.

during the National Marine Fisheries Service (NMFS) Tiburon Groundfish Communities Investigation surveys in May and June (Adams, 1992).

The May–July 1993 study period was characterized by sporadic, strong winds, mostly from the south (downwelling) in May and from the northwest (upwelling) in June and July (Figure I). Juvenile rockfish were collected during three prolonged surveys spaced about 2 weeks apart with several 1- to 3-day efforts within each survey.

Survey 4 (May 5–12) was conducted north of Monterey Bay. Cool, relatively saline surface water (Table 3) suggested the influence of upwelling off Pescadero Point (as indicated by satellite images during April). Only 37 rockfish were collected in 11 tows (Figure 11A). Survey 5 (May 25–June 6) was conducted south of Monterey Bay. On average, the temperature in the upper water column was 3°C warmer and salinity was 0.36 ppt lower than those north of the bay during survey 4 (Table 4). This likely reflected the onshore influence of a warm-water meander off Monterey Bay (Figure 5). A total of 988 rockfish were collected in 17 samples, with catch sizes ranging from 1 to 240 per tow (Figure 11B). Survey 6 (June 20–July 9) was conducted both north and south of the bay. Decreased temperature and increased salinity in the southern upper water column, relative to those in the previous survey, suggest decreased influence by the meander and possibly some influence of upwelling off Point Sur. Relatively high salinity to the north also indicates upwelling off Pescadero Point. Twenty tows north of the bay contained 515 rockfishes. A total of 4842 rockfishes were collected in 13 tows made to the south (Figure 11C). Tows with catches of 1480 and 1774 rockfishes were made within 15 m of the bottom nearshore off Cypress Point (60-m bottom depth) and Point Piños (65-m bottom depth). Another catch of 1312 rockfishes was located within 25 m of the bottom at Portuguese Ledge, a shallow rock outcrop off Point Piños (Figure 11C). The association between these three large catches and shallow water suggests that we likely sampled near-bottom aggregations rather than pelagic juvenile rockfishes.

A total of 25 rockfish taxa were collected in 1993 (Table 4). Shortbelly rockfish was again the dominant species, accounting for 4809 (75%) of the total 6382 rockfish collected. Other relatively abundant species included squarespot (*S. hopkinsi*) and halfbanded (*S. semicinctus*) rockfish. Almost all taxa occurred more often and were more abundant in samples collected south of the Bay (Table 4). Excluding the three large, probably near-bottom catches, the overall mean abundance in southern samples was more than double that of the northern samples. A similar pattern occurred in 1992 (Table 5).

In 1993 juvenile rockfishes were 20 times more abundant, included twice as many taxa, and were substantially larger than in 1992 (Table 4; Figures 10C, and 10D). A growth model established for

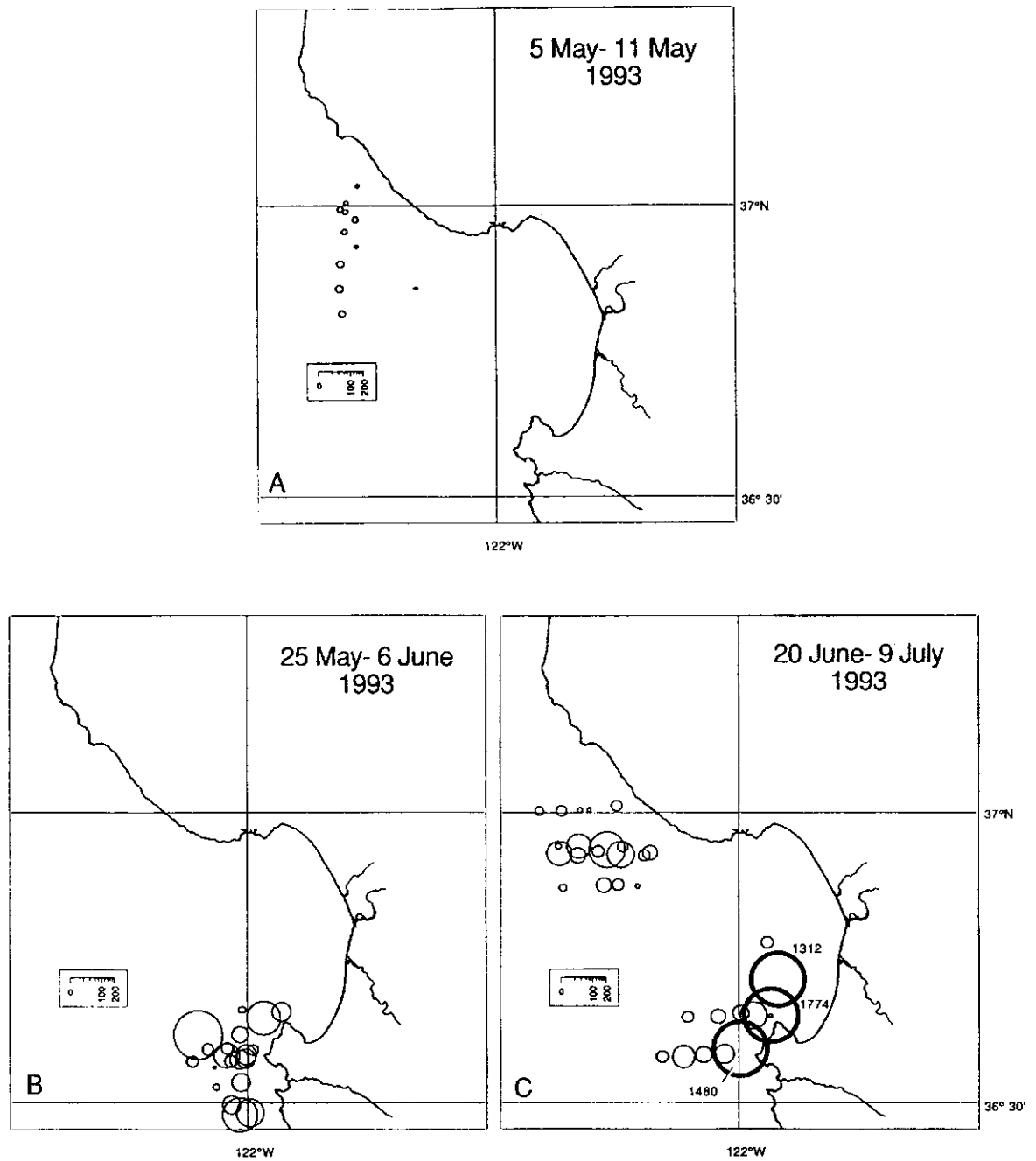

Figure 11. Location of net tows and abundance of pelagic juvenile rockfishes collected in 1993. A, Survey 4 (5–11 May). B, Survey 5 (25 May–6 June). C, Survey 6 (20 June–9 July).

Table 4. Pelagic Juvenile Rockfishes Collected During Midwater Trawl Surveys in the Vicinity of Monterey Bay, May–July 1992 and 1993

Common Name	Species	1992 Total	1992 (56 Trawls) Mean No./Trawl	1992 %	1993 Total	1993 (64 Trawls) Mean No./Trawl	1993 %
Shortbelly	*S. jordani*	131	2.34	56.5	4809	73.98	75.4
Copper complex		43	0.77	18.5	122	1.88	1.9
Kelp	*S. atrovirens*	17	0.30	7.3	–	–	–
Chilipepper	*S. goodei*	12	0.21	5.2	143	2.20	2.2
Brown	*S. auriculatus*	12	0.21	5.2	95	1.46	1.5
Bocaccio	*S. paucispinis*	4	0.07	1.7	41	0.63	0.6
Splitnose	*S. diploproa*	4	0.07	1.7	1	0.02	0.0
Squarespot	*S. hopkinsi*	3	0.05	1.3	493	7.58	7.7
Stripetail	*S. saxicola*	2	0.04	0.9	35	0.54	0.5
Sebastomus group		2	0.04	0.9	13	0.20	0.2
Sp. B		1	0.02	0.4	–	–	–
Sp. A		1	0.02	0.4	–	–	–
Halfbanded	*S. semicinctus*	–	–	–	463	7.12	7.3
Widow	*S. entomelas*	–	–	–	74	1.14	1.2
Blue	*S. mystinus*	–	–	–	29	0.45	0.5
Yellowtail	*S. flavidus*	–	–	–	19	0.29	0.3
Olive	*S. serranoides*	–	–	–	9	0.14	0.1
Darkblotched	*S. cramen*	–	–	–	9	0 14	0 1
Canary	*S. pinniger*	–	–	–	4	0.06	0.1
Grass	*S. rastrelliger*	–	–	–	3	0.05	<0.1
Greenstripe	*S. elongatus*	–	–	–	3	0.05	<0.1
Unid. 1		–	–	–	2	0.03	<0.1
Black	*S. melanops*	–	–	–	2	0.03	<0.1
Cowcod	*S. levis*	–	–	–	1	0.02	<0.1
Unid.5		–	–	–	1	0.02	<0.1
Unid. 4		–	–	–	1	0.02	<0.1
Bank	*S. rufus*	–	–	–	1	0.02	<0.1
Unid.7		–	–	–	1	0.02	<0.1
Unid.		1	0.02		8	0.12	
Total		233	4.16		6382	98.18	
No. taxa		12			25		

Note: Copper complex includes copper (*S. caurinus*), gopher (*S. carnatus*), and black-and-yellow (*S. chrysomelas*) rockfish. *Sebastomus* group includes greenspotted (*S. chlorostictus*), starry (*S.* constellatus), swordspine (*S. ensifer*), pink (*S. eos*), rosethorn (*S. helvomaculatus*), rosy (*S. rosaceous*), and greenblotched (*S. rosenblatti*) rockfish. Sp. = species. Unid. = unidentified. Dashes = 0 collected.

Table 5. Pelagic Juvenile Rocktishes Collected During Midwater Trawl Surveys in Southern and Northern Monterey Bay Areas May–July 1992 and 1993

	South				North			
	1992							
Taxon	(30 Trawls) Total	Mean No./Trawl	S.D.	Frequency (%)	(26 Trawls) Total	Mean No./Trawl	S.D.	Frequency (%)
Shortbelly	112	3.73	7.54	33.0	19	0.73	1.40	31.0
Copper complex	26	0.87	1.82	30.0	17	0.65	1.54	27.0
Kelp	14	0.47	1.09	23.0	3	0.12	0.42	8.0
Chilipepper	11	0.37	1.05	13.0	1	0.04	0.19	4.0
Brown	9	0.30	0.78	17.0	3	0.12	0.32	12.0
Splitnose	4	0.13	0.43	10.0	–	–	–	–
Bocaccio	4	0.13	0.43	10.0	–	–	–	–
Squarespot	2	0.07	0.25	7.0	1	0.04	0.19	4.0
Sebastomus group	2	0.07	0.25	7.0	–	–	–	–
Stripetail	1	0.03	0.18	3.0	1	0.04	0.19	4.0
Unid. A	1	0.03	0.18	3.0	–	–	–	–
Unid. B	1	0.03	0.18	3.0	–	–	–	–
Unid. damaged	1	0.03	0.18	3.0	–	–	–	–
Total	188	6.27	11.13	50.0	45	1.73	2.89	46.0
No. taxa	12	1.60	2.00		7	0.90	1.10	

	1993							
Species	(30 Trawls) Total	Mean No./Trawl	S.D.	Frequency (%)	(31 Trawls) Total	Mean No./Trawl	S.D.	Frequency (%)
Shortbelly	735	24.50	39.21	96.7	368	11.87	24.07	58.1
Chilipepper	100	3.33	6.30	63.3	32	1.03	1.33	48.4
Widow	73	2.43	5.73	30.0	–	–	–	–
Copper complex	66	2.20	4.81	40.0	43	1.39	2.96	45.2
Squarespot	64	2.13	4.57	43.3	21	0.68	1.45	29.0
Bocaccio	37	1.23	2.84	40.0	3	0.10	0.39	6.5
Halfbanded	18	0.60	2.54	10.0	10	0.32	0.82	16.1
Brown	32	1.07	1.67	43.3	61	1.97	3.21	67.7
Stripetail	29	0.97	2.54	30.0	4	0.13	0.42	9.7
Blue	28	0.93	1.95	30.0	1	0.03	0.18	3.2
Yellowtail	17	0.57	1.09	26.7	1	0.03	0.18	3.2
Sebastomus group	12	0.40	1.38	10.0	–	–	–	–
Darkblotched	9	0.30	0.69	20.0	–	–	–	–
Olive	8	0.27	0.57	20.0	1	0.03	0.18	3.2
Canary	4	0.13	0.34	13.3	–	–	–	–
Greenstripe	3	0.10	0.40	6.7	–	–	–	–
Black	1	0.03	0.18	3.3	1	0.03	0.18	3.2
Unid. 4	1	0.03	0.18	3.3	–	–	–	–
Unid. 7	1	0.03	0.18	3.3	–	–	–	–
Bank	1	0.03	0.18	3.3	–	–	–	–
Cowcod	1	0.03	0.18	3.3	–	–	–	–
Unid. 5	1	0.03	0.18	3.3	–	–	–	–
Grass	–	–	–	–	3	0.10	0.53	3.2
Unid.1	–	–	–	–	2	0.06	0.35	3.2
Splitnose	–	–	–	–	1	0.03	0.18	3.2
Unidentitied	6	0.18	0.46	16.7	–	–	–	–
Total	1247	41.37	47.57	100.0	552	17.81	28.74	96.8
No. taxa	22	5.43	2.86		15	3.03	1.69	

Note: Data from southern Monterey Bay 1993 do not include three extremely large, probably near-bottom catches. SD = standard deviation. Unid. = unidentified. Dashes = 0 collected.

shortbelly rockfish juveniles collected off central California during the 1983 El Niño (Woodbury and Ralston, 1991), was used to estimate age, and hatch dates were calculated. Surviving juvenile shortbelly rockfish were born late in the season during both years (median hatch dates, April 21, 1992 and March 31, 1993; Figure 8). Upwelling occurred coincidentally during March and April in 1993, but not in 1992. Limited information, indicated high numbers of chaetognaths, a potential predator collected in the ichthyoplankton samples, nearshore during peak abundance of larval rockfish in February 1992 (J. Bridges, unpublished data). Our data indicate substantially higher survival and recruitment of rockfish in 1993; we speculate that this is due to increased upwelling and offshore transport, lower predation, and possibly better feeding conditions during the larval stages.

Discussion

In accordance with the results of our surveys, subtidal observations along the central coast made by biologists of the CDFG Sportfish Project indicated that few juveniles of nearshore rockfish species settled into rocky reef and kelp canopy areas off the Monterey Peninsula during May–August 1992 (Ven Tresca, unpublished data). Overall rockfish recruitment in 1992 was the lowest estimated over their 1990–1993 study period. Relatively high numbers of rockfish settled during June–September 1993, indicating successful recruitment of the pelagic juveniles that we sampled earlier in the year. Subtidal recruitment levels in 1993 were similar to those during 1990, a non–El Niño period.

The low abundance and small size of juvenile rockfishes off central California during 1992 also were noted during the May–June 1992 Groundfish Communities Investigation surveys (Ralston, 1993) and were characteristic of previous El Niño years (Wyllie Echeverria et al,. 1990). However, the larger sizes and higher abundance of the pelagic juveniles during the continued El Niño in May–July 1993 suggest that poor recruitment success is not just the consequence of an El Niño event but is most likely also related to the timing of optimal hydrographic conditions occurring during the larval and postlarval stages.

Net onshore transport during the peak abundance of larval rockfish in February 1992 could have been responsible for heavy larval mortality, most likely through increased predation relative to that in offshore waters. Because of the relatively low intensity of the upwelling and the lack of offshore transport during the subsequent months, possible prolonged nearshore retention and elevated predation of the postlarval stages and juveniles further reduced recruitment success. In contrast, increased intensity of upwelling and offshore transport during March and April 1993 likely facilitated survival of larval and postlarval stages by advection from nearshore areas and by increased densities of prey.

High diversity and abundance of juvenile rockfish in southern Monterey Bay during 1993 may have been associated with the warm offshore meander observed during May and June. Possible transport of offshore water to nearshore southern Monterey Bay via this hydrographic feature could have facilitated recruitment and settlement. This feature could combine young rockfishes that were transported offshore from northern source areas with rockfishes from southern source areas that were transported onshore along the coast.

Collaboration and Dissemination of Information

Because we have taken an interdisciplinary approach to understanding processes that affect recruitment of young rockfishes to nearshore areas in Monterey Bay, our study included the efforts of physical oceanographers and fishery scientists from several regional institutions. Personnel from the CDFG Sportfish Project conducted subtidal surveys of rockfishes to evaluate nearshore benthic recruitment into Monterey Bay; this perspective was important in evaluating our sampling program and results. CDFG supplied fisheries personnel, charter vessel time, and equipment; our results are directly applicable to their ongoing subtidal recruitment studies and are critical to the management and monitoring of future resource reserves.

Personnel from the NMFS Tiburon Groundfish Communities Investigation have provided unpublished information on the abundance of young-of-the-year rockfishes collected in and around Monterey Bay during surveys in May and June 1991, 1992, and 1993. This was helpful in preparation for our pelagic rockfish cruises and interpretation of results. They also helped confirm identification of juvenile rockfish species in our samples. The NMFS Pacific Fisheries Environmental Group provided timely estimates of daily and weekly upwelling index and personnel for shipboard operations; Frank Schwing has advised us on interpretation of the physical oceanographic data. About 50 near-real-time AVHRR satellite images and appropriate software were made available by Mike Laurs (NMFS, Southwest Fisheries Science Center) to assist in locating our May–July sampling stations.

A Consortium of Monterey Bay Regional Oceanographic Studies was established by M. Yoklavich and F. Schwing. Four informal meetings were held and were attended by about 20 scientists from Moss Landing Marine Laboratories, NMFS Pacific Fisheries Environmental Group, NMFS Tiburon Groundfish Communities Investigation, Monterey Bay Aquarium Research Institute, CDFG, University of California, Santa Cruz, and Naval Postgraduate School. These meetings provided an opportunity to exchange details of sampling plans and results and encouraged collaboration among institutions and research groups with compatible interests in Monterey Bay.

Two workshops on identification of larval and juvenile rockfishes were convened by our project personnel and were attended by researchers from NMFS Tiburon, CDFG, and San Francisco State University.

Cooperating Organizations

California Department of Fish and Game Sportfish Project, Monterey
Moss Landing Marine Laboratories
National Marine Fisheries Service, Pacific Fisheries Environmental Group
National Marine Fisheries Service, Southwest Fisheries Science Center
National Marine Fisheries Service, Tiburon Groundfish Communities Investigation

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Control of Growth and Survival in Striped Bass

University of California, Berkeley
R/F-145
1993–95

Howard A. Bern and Richard S. Nishioka (with Steffen Madsen, Christopher Loretz, Kimmakone Siharath, Karen Lee, Jason Datuin, Melanie Kim, Tamy Brown, Richard Park, and Claus Brigmann)

We have continued our studies of the endocrine regulation of growth in striped bass (*Morone saxatilis*) with the intent of completing all studies that we initiated. Some studies are in various stages of completion and are reported herein in preliminary form.

In addition to growth hormone, insulin-like growth factors (IGFs) play a major role in the growth of striped bass. Proteins that bind to IGFs (IGFBPs) are important in regulating the availability of and responses to these factors. Striped bass appear to have four IGFBPs in circulation. Three of these (IGFBP-1, IGFBP-2, and IGFBP-3) are similar to IGFBPs found in mammals.

In order to determine the tissue source of binding proteins, various organs of striped bass were cultured. Organs were removed and cultured in minimum essential medium for 48 hours in an environment of 95% oxygen/5% carbon dioxide at 16°C. Protein content of the medium was determined by using a protein assay kit, and the same amounts of protein were analyzed by using 12.5% SDS-polyacrylamide gel electrophoresis. After the proteins were transferred to nitrocellulose sheets, radiolabeled human IGF-I was used to detect the presence of striped bass IGFBPs. Culture medium from liver contained both IGFBP-1 and IGFBP-2, whereas culture media from the kidney, bulbus arteriosus, and pituitary contained only IGFBP-2. Media from other sources such as optic lobes, spleen, gonad, gill filaments, ceratobranchial cartilage, ceratobranchial bone, heart, muscle, and gut gave inconsistent results; however, when detectable, IGFBP-2 seemed to be the only striped bass IGFBP in the medium. Multiorgan production of IGFBP appears to be an evolutionarily conserved mechanism in vertebrates, evident in both teleosts and mammals.

Using the same *in vitro* system, we have investigated the hormonal regulation of striped bass IGFBPs. Liver cubes from striped bass were exposed to various hormones and growth factors. Ovine prolactin, insulin, and recombinant bovine IGF-I all reduced the amount of striped bass IGFBP-1 present in the medium. Addition of estradiol, however, increased production of this striped bass binding protein. The amount of striped bass IGFBP-2, on the other hand, was decreased by glucagon, thyroxine, and human IGF-I and increased by ovine growth hormone, estradiol, and human epidermal growth factor. These results indicate that IGFBPs in striped bass are hormonally controlled.

The role of growth hormone in the regulation of IGFBP in striped bass was examined in hypophysectomized, sham-operated, and intact fish. Hypophysectomy did not consistently change the serum levels of IGFBP-1 and IGFBP-2. However, it significantly decreased serum levels of IGFBP-3. Injection of ovine growth hormone at a concentration of 2 μg per gram of body weight or tilapia growth hormone at a concentration of 2 μg/g restored the levels of striped bass IGFBP-3 to those seen in sham-operated fish. Injection of ovine growth hormone also increased levels of striped bass IGFBP-4 in hypophysectomized fish. In intact striped bass, injection of ovine growth hormone at a concentration of 2 μg/g did not significantly change the serum profile of IGFBPs. On the other hand, treatment with ovine growth hormone stimulated uptake of ^{35}S-sulfate by ceratobranchial cartilage in both the hypophysectomized and intact fish. The presence of IGFBPs in the serum of striped bass and the response to hypophysectomy and subsequent replacement with heterologous growth hormone suggest an evolutionary conservation of the growth hormone–IGF-IGFBP axis.

In addition, the effect of hypophysectomy on the ability of striped bass to adapt from one-third seawater to either fresh water or seawater was investigated. Earlier experiments had shown that hypophysectomized striped bass can survive in seawater and in one-third seawater, but not in fresh water because of osmoregulatory failure. In the experiment described here, fish were hypophysectomized and allowed to recover in one-third seawater for 7 days. After recovery, the fish were given multiple injections of ovine prolactin or recombinant striped bass growth hormone at two dose levels. After the last injection, groups of fish were transferred either to fresh water or to seawater and sampled 24 hours later. Both prolactin and growth hormone appeared to abolish in part the osmoregulatory disturbance of hypophysectomized fish transferred to fresh water. However, on the basis of measurements of plasma osmolality, prolactin was somewhat hyperosmoregulatory, and growth factor was slightly hypoosmoregulatory. We are currently measuring plasma ion levels and gill and kidney sodium, potassium-ATPase activity and expression and are using electron microscopy to examine the morphology of gill chloride cells. These studies will help us determine the extent to which prolactin and growth hormone are involved in freshwater and seawater adaptation in striped bass.

The effect of a single injection of recombinant bovine IGF-I (Monsanto) at two doses or insulin (one dose) was examined in a 24-hour seawater challenge test. Treatment with IGF-I deleteriously affected

acclimation to seawater by increasing plasma osmolality and reducing muscle water content. Insulin did not affect plasma osmolality but did reduce muscle water content. Thus, the two hormones do not favor acclimation to seawater in striped bass.

Striped bass were either fed or fasted for a total of 60 days and then refed for 14 days. Samples were collected on days 30 and 60 during the fasting period and on day 14 after refeeding. Fasting caused a time-dependent increase in serum levels of striped bass IGFBP-1 that were significantly higher than the serum levels in fed controls collected at the same time points. Refeeding the fasted fish decreased IGFBP-1 levels to those observed in the fed controls. Production *in vitro* of striped bass IGFBPs from the liver, spleen, and kidney of the same fish, however, was not affected by fasting. Uptake of ^{35}S-sulfate and ^{3}H-thymidine by gill cartilage was significantly lower by day 30 of fasting but did not further decrease by day 60. This decrease was reversed by 14 days of refeeding. Fasting also caused a decrease in the growth rate. Thus, food deprivation profoundly affects the IGF-IGFBP axis, which may be linked to the decrease in proteoglycan synthesis and mitotic activity in cartilage, and consequently in whole body growth.

Receptors for IGF were found in the brain of striped bass in a preliminary study. Administration of recombinant striped bass growth hormone caused a decrease in receptor binding in the brain. However, both administration of bovine growth hormone and feeding resulted in increased binding. Possibly, bovine growth hormone is not as effective as recombinant striped bass growth hormone in initiating production of IGF-I and therefore allows greater binding to receptors, instead of down-regulating them.

These physiological studies on the growth hormone–IGF-IGFBP axis are intended to provide the basis for decisions on the possible endocrine manipulation of striped bass to improve growth and survival of the fish in aquaculture and hatchery operations. The studies are also intended to help in understanding of the survival of the fish under natural conditions in Northern California, where the population of striped bass has become increasingly threatened.

Addendum

As an integral part of our interest in the hormonal control of growth in fishes and the role of IGF-I therein, we studied receptors for IGF-I in striped bass. The presence of the receptors and some of the factors influencing their concentration were examined in brain, spinal cord, and liver. Both growth hormone and nutrition appear to modulate the level of IGF-I receptors. Changes in the level were detected in fed fish only. In the brain, specific binding decreased significantly from 7.3% to 2.5% after injection of recombinant striped bass growth hormone (provided by T.T. Chen). In the liver and the spinal cord, however, binding increased significantly, from 4.6% to 6.6% and from 4.0% to 7.3%, respectively. The discrepancy between the brain and the spinal cord is interesting: the brain may be protected from growth hormone/IGF-I effects by a reduction in IGF-I binding capacity. Studies of IGF-I receptor biology are being extended to tilapia.

We continued an investigation of the effect of hypophysectomy on the ability of striped bass held in one-third seawater to adapt to either fresh water or seawater. The results confirmed our preliminary data that hypophysectomized fish can survive and adapt to seawater and one-third seawater but not to fresh water. Replacement therapy was carried out with multiple injections of either ovine prolactin or recombinant striped bass growth hormone. Both hormones appeared to minimize osmoregulatory disturbance in hypophysectomized fish in fresh water. In hypophysectomized fish in seawater, prolactin is hyperosmoregulatory, and growth hormone is weakly hypoosmoregulatory. In fish kept in one-third seawater, hypophysectomy induced an increase in sodium, potassium-ATPase activity that was abolished when the fish were transferred to fresh water or to seawater. Low doses of growth hormone simulated gill enzyme activity in fish transferred to fresh water or seawater. Morphological examination of gill chloride cells is under way. In addition, gills will be analyzed for expression of mRNA for the α-subunit of sodium potassium-ATPase.

A 24-hour seawater challenge was used to investigate the effect of recombinant bovine IGF-I and insulin. The osmoregulatory data confirmed our previous observation: IGF-I adversely affects acclimation to seawater by increasing plasma osmolality and reducing muscle water content. This nonadaptive effect of IGF-I in striped bass exposed to seawater stands in contrast to observations made earlier on salmonids. Insulin did not affect plasma osmolality but did reduce muscle water content.

Cooperating Organizations

Bodega Marine Laboratory
California Department of Fish and Game
Monsanto Company, St. Louis, Missouri
University of Maryland, College Park, Maryland

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Radiometric Age Verification of Commercially Important Deep-Water Fishes

Moss Landing Marine Laboratories
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1993–95

Gregor M. Cailliet, Kenneth H. Coale, and Allen Andrews

Deep-water fishes, such as bank rockfish (*Sebastes rufus*), yelloweye rockfish (*Sebastes ruberrimus*), thornyhead rockfishes (*Sebastolobus altivelis* and *Sebastolobus alascanus*), and two species of grenadier (*Coryphænoides acrolepis* and *Albatrossia pectoralis*), are being commercially harvested at an increasing rate off the western coast of the United States (Jacobson, 1991; Hosie and Stein, 1992). However, estimates of the age composition, growth, age at maturity, and longevity of these fishes have not been verified. Estimates of age based on growth zones in otoliths from these species suggest that the fish are long-lived and could be vulnerable to heavy fishing pressures (Matsui et al., 1990; Fenton et al., 1991; Jacobson, 1991; Ianelli et al., 1994)

The basic objective of this study was to show the feasibility of using the radiometric age-determination approach (Bennett et al., 1982; Fenton et al., 1990; Campana et al., 1990) in bank and thornyhead rockfishes and to extend this approach to other deep-water fishes.

Radiometric results indicate that the method works on the otoliths of these rockfishes (Kline et al., 1993; Watters et al,. 1993). We accomplished this objective by (1) establishing the capability of using preparation and handling techniques that introduced no contaminating trace metals (i.e., trace-metal-clean), a requirement for radiochemical analyses of samples with low radioactivity, (2) calibrating both the α-spectrometer and the radon emanation system for use with anticipated activity levels, (3) developing the techniques and local capability of plating polonium-210 (daughter/proxy for lead-210) and determining ^{210}Po with α-spectrometry, (4) measuring the background activity of the two systems that will be used and the backgrounds for all chemicals and preparation technique blanks, (5) preparing otolith samples for radiometric analyses, and (6) doing the radiometric analysis and plotting results against age estimates based on examinations of sectioned otoliths (Chilton and Beamish, 1982).

Other successful radiometric analyses of fish otolith material have emphasized that because of the very low radioactivity of the isotopes in the otoliths, clean techniques must be used during preparation of samples (Bennett et al., 1982; Campana et al., 1990; Fenton et al., 1990; Kastelle, 1991; Smith et al., 1991; Jones, 1993; Kastelle et al., 1994). Therefore, we developed a protocol for preparation and cleaning that was based on trace-metal techniques (Fabry and Delaney, 1989; Linn, 1988) and established procedures for preparing otoliths (Bennett et al., 1982; Campana et al., 1990).

This protocol consists of coring each otolith and combining cores of similar ages. The cores are then cleaned repeatedly with acid and Millipore®-filtered deionized water (18 M Ω cm^{-3}) to remove the potentially contaminated surface layer of the core. Samples are kept free of dust, and acid-cleaned equipment is used for handling and storage. Cleaned core samples are dissolved and plated by using the ^{210}Po-plating procedure. These ^{210}Po samples are analyzed as a proxy for ^{210}Pb activity.

Samples prepared for ^{210}Po analysis are spiked with a yield tracer (polonium-208). The ^{210}Po and tracer are autodeposited on a silver planchet (Flynn, 1968) and radioactivity is determined with an α-spectrometer. The α-spectrometer system consists of eight Tennelec TC256 α-spectrometers with multichannel analyzer and an eight-channel digital multiplexer with Nucleus software on an IBM personal computer that has been constructed and calibrated at our laboratory facilities. Because the amount of radioactivity in the samples is very low for lead (e.g., 83–550 μBq/g, where 1 μBq = 10^{-6} disintegrations/sec), more than 3 weeks is required for sufficient counts to be acquired. We have defined ^{208}Po and ^{210}Po channel intervals for each of the eight detectors that make up the α-spectrometer system. Background activity for each detector has been counted. This was done by (1) adjusting the bias voltage to allow the detection of a 4 to 6-Mev range of α-emitters, (2) counting standards of known isotopes (^{238}U, ^{234}U, ^{232}U, ^{232}Th, ^{230}Th, ^{228}Th, ^{208}Po, ^{210}Po) on all eight detectors, (3) determining energy-specific channels in each detector and using the results from standards, (4) counting planchets containing only ^{210}Po and ^{208}Po, and (5) making background counts (with reagent blanks on silver planchets in the detectors) for ^{210}Po and ^{208}Po.

Use of trace metal-clean reagents and procedures is important in estimating these low levels of radioactivity. We have tested our clean preparation technique, fine-cleaning reagents, and dissolution acid for lead contamination by plating them in the same manner that samples are plated and determining radioactivity with the α-spectrometer. We found that the ^{210}Po and ^{208}Po activity in these samples was only slightly above background for the α-spectrometer system used. As an extra precaution, we constructed a trace metal-clean positive-pressure hood for use in polonium plating. The hood further prevents possible contamination from atmospheric sources, where lead can be a real problem,

and has provided a clean workplace for the new radium separation technique.

In all previous ^{210}Pb: radium-226 disequilibria ageing studies, ^{226}Ra activity was determined by using the radon-222 emanation technique. This process requires about 4–5 weeks to obtain results from one sample. At first, we tried to use a technique developed by Volpe et al. (1991) and Cohen and O'Nions (1991) that was modified by geochemists at University of California, Santa Cruz (UCSC), to measure ^{226}Ra directly in geological samples. Initial attempts to use this method with the otolith matrix ultimately failed. After several attempts to modify the procedures, we directed our attention to a simpler approach (Chabaux et al., 1994).

After much research and development in applying the new approach to the otolith matrix, we designed a new technique to separate radium from the other components of the otolith. This technique involves separating radium from calcium and barium by using a series of cation-exchange columns. Calcium and barium must be removed because their presence in high quantities suppresses the radium signal when a sample is analyzed on a thermal ionization mass spectrometer (TIMS). Once the calcium and barium are reduced to levels that do not suppress the radium signal, radium can be measured on the TIMS directly.

The time required to process several samples is about 7–10 days, and the amount of material required (0.5–1.0 g) is about one-tenth the amount usually required for the radon emanation technique. Because only 0.5 –1.0 g of otolith material is required, the same sample can be used for both ^{210}Pb analysis and ^{226}Ra analysis. The attempt to perfect this technique for otolith material, done at UCSC in collaboration with Xenon Palacz, director of the Mass Spectrometer Facility, and graduate student Craig Lundstrom, has lead to an independent development of the separation technique for otolith material.

The new and simplified technique designed by Chabaux et al. (1994) to separate radium from volcanic rocks was used as a guideline for our experimental design. For the first column separation, the design is the same as that of Chabaux et al. (1994). In order to analyze the separation capability of this arrangement for calcium and radium, elution curves were constructed by using an atomic absorption spectrophotometer and barium as an analog for radium, because these two elute together. Separation of calcium and barium was good for 1- to 2-g samples. However, a small amount of overlap between the calcium and barium elution makes it necessary to collect a small part of the calcium fraction. This small part contains approximately 5% of the total calcium. Therefore, a second separation through the same column is necessary to remove most of the calcium and conserve any of the barium (and presumably radium) in the overlap. The calculated removal of calcium from the sample after use of the first two columns is 99.75%. However, 0.25% may still cause interference on TIMS analysis.

The final separation column removes barium from radium. Analysis of elution curves available from the manufacturer indicated that the resin will also remove calcium at certain concentrations of acid. We selected the concentration used by Chabaux et al. (3.0N HNO_3) and another (1.1N HNO_3) that would optimize the separation between calcium, barium, and radium. Because radium will elute between calcium and barium and cannot be detected with our atomic absorption spectrophotometer, we also attempted to maximize the separation between the calcium and barium peaks. The results of this experiment indicated that approximately 100% of the calcium was separated from the barium and that the widest separation between the calcium and barium peaks occurred with the selected acid concentration (1.1N HNO_3). The next step was to use the TIMS to analyze the region between these peaks for radium.

To test the new separation procedure, we analyzed otoliths from a variety of fish species (Table 1). Each of these species is being investigated on an individual basis with the radiometric age-verification technique. Both whole and cored otoliths have been used, for total sample weights of 0.303–1.333 g. The largest samples were whole otolith samples from *C. acrolepis* and *S. alascanus*. The smallest samples were whole and cored otolith samples from *Megalops atlanticus* and *S. ruberrimus*. Samples of *C. acrolepis* were otolith pairs from individual fish. Samples of *S. alascanus* were pooled whole otoliths from several adults. The result for this species is unique, because it can be compared with results from similar samples of the same species (Kline, unpublished data). Although ^{222}Rn emanation and TIMS results are lower but comparable (0.0387–0.0504 disintegrations per minute [dpm]/g and 0.03176 dpm/g, respectively), direct α-spectrometry gave different results. It remains to be determined why this difference occurred. The samples of *M. atlanticus* were pooled young-of-the-year collected from various locations in Florida. These results are part of a feasibility study to determine if radiometric age verification is applicable. The samples of *S. ruberrimus* were pooled whole and cored otolith samples. Analyses of these samples showed several important concepts associated with this new method. First, the sample sizes were very small and had low ^{226}Ra activity (0.02514–0.03268 dpm/g). This finding shows that the method can be used to detect ^{226}Ra in smaller samples than those previously used and with higher accuracy. Second, the cored samples were analyzed for ^{210}Pb before they were analyzed for ^{226}Ra. Hence, the same small sample can be used for both analyses. Although core samples with a total weight of 1 g were used by Kastelle et al. (1994) for both analyses, higher precision and accuracy can be attained with the TIMS method (Cohen and O'Nions, 1991; Volpe et al., 1991).

An additional advantage is that when the same core sample is used for ^{210}Pb and ^{226}Ra analyses, the calculation of the core age is independent of the sample weight. Hence, uptake of radium does not

Table 1. List of Otolith Samples Processed for TIMS Determination of ^{226}Ra Activity

Sample Number	Species	Sample Weight (g)	Whole/Core	^{228}Ra Spiked	^{226}Ra Activity (dpm/g)
MLML1	*S. alascanus*	1.333	Whole	No	N/A
MLML2	*C. acrolepis*	1.2606	Whole	No	N/A
MLML3	*M. atlanticus*	0.8081	Whole (YOY)	No	N/A
MLML4	*S. ruberrimus*	0.9165	Whole	No	N/A
MLML5	*S. ruberrimus*	0.8819	Whole	Yes	0.02591
MLML6	*M. atlanticus*	0.4506	Whole (YOY)	Yes	0.17227
MLML7	*S. alascanus*	1.2607	Whole	Yes	0.03176
MLML8	*C. acrolepis*	1.2826	Whole	Yes	0.04136
MLML9	*C. acrolepis*	1.2799	Whole	No	Lost
MLML10	*M. atlanticus*	0.5068	Whole (YOY)	Yes	0.19881
MLML11	*M. atlanticus*	0.4798	Whole (YOY)	No	N/A
MLML12	Spiked blank	Blank	N/A	Yes	N/A
MLML13	*S. ruberrimus*	0.319	Core	Yes	0.02514
MLML14	*S. ruberrimus*	0.303	Core	Yes	0.03008
MLML15	*S. ruberrimus*	0.471	Core	Yes	0.02939
MLML16	*M. atlanticus*	0.564	Whole (YOY)	Yes	1.62764
MLML17	*M. atlanticus*	0.584	Whole (YOY)	No	N/A
MLML18	Spiked blank	Blank	N/A	Yes	N/A
MLML19	*S. ruberrimus*	0.587	Core	Yes	0.02919
MLML20	*S. ruberrimus*	0.592	Core	Yes	0.02804
MLML21	*S. ruberrimus*	0.561	Core	Yes	0.03268

Note: All *M. atlanticus* samples were young-of-the-year (YOY). For samples to which no ^{228}Ra spike was added, detection of ^{226}Ra activity was not applicable (N/A). These runs were used to determine the amount of naturally occurring ^{228}Ra. Sample MLML9 was lost for unknown reasons, and samples MLML18 and MLML19 may have been switched.

need to be constant relative to mass growth, and the amount of radium between cores in the sample does not need to be the same. This situation alleviates the need to assume that the activity of ^{226}Ra in whole otoliths (usually used to get enough mass for radium analysis) is not significantly different compared with the activity in the core. For example, in the worst-case scenario for a 3-year core sample, all the ^{226}Ra would be deposited in the third year. This event would cause the age estimate to be underestimated by 2–3 years. Therefore, this effect becomes less significant for younger cores and older fish.

In general, the ^{226}Ra activities of the otoliths used are in the range of values reported by all the previously cited sources (0.006–0.460 dpm/g). The exception is one *M. atlanticus* sample (1.6276 dpm/g). Nearshore waters, where the juveniles of this species spend their first year, may be variable in ^{226}Ra content. Hence, elevated ^{226}Ra levels at the site where these fish were taken may be the cause of elevated levels of ^{226}Ra in the otoliths.

Otoliths collected for each fish species are the best size range that was attainable. For some fish species, we have few otoliths in the very small and very large fish sizes. However, we think that the samples collected are enough to adequately describe and verify the growth characteristics of each fish species. The one exception is *A. pectoralis*, for which we are still obtaining a representative set of otoliths for analysis. Otoliths collected for each fish species have been processed and aged by using traditional techniques. With the exception *of A. pectoralis*, growth characteristics have been developed on the basis of the age estimates for each fish species. However, otoliths from medium-sized *A. pectoralis* have been sectioned, and the results indicate that the otoliths are useful for estimating age.

Except for the final determination of ^{226}Ra activity, the preparation and processing techniques used for radiometric age determination are well refined and are used in our laboratory. The last determination has been done on the TIMS at UCSC. The preparation and processing techniques used for the determination of ^{226}Ra activity have evolved over time; therefore, the techniques used were different among the fish species analyzed. The ^{222}Rn emanation technique as described by Bennett et al. (1982) was used for the first three species analyzed (*S. altivelis*, *S. alascanus*, and *S. rufus*), whereas the new chromatographic separation technique was used to assess ^{226}Ra activity in the otoliths of *S. ruberrimus*, *C. acrolepis*, and *M. atlanticus*, and in a follow-up test for *S. alascanus*.

In most cases, radiometric age estimates were calculated and compared with traditional ageing estimates for each fish species. For *S. rufus*, traditional age estimates closely correlate with radiometric age estimates and indicate a longevity of at least 40 years. These results are being refined for publication (Watters, 1993). The *Sebastolobus* species have been more of a problem because of differences in the measured ^{226}Ra activity. Although the ^{222}Rn emanation technique was initially used to determine ^{226}Ra activity, a follow-up determination with a third ^{226}Ra assessment technique (Fenton et al., 1990) produced conflicting results for *S. alascanus*. However,

the new ^{226}Ra assessment technique produced results that support the results of ^{222}Rn emanation. A future project may explore the reasons for the differences in these results. On the basis of the TIMS ^{226}Ra values, verified longevity estimates are at least 80 years for *S. alascanus* and at least 45 years for *S. altivelis*.

Sebastes ruberrimus, a recent addition to the project, has led to rapid and final results. Preliminary radiometric age estimates suggest close agreement with age estimates based on otolith sections and break-and-burn methods, and may indicate that the longevity of this species is at least 100 years. This would make the yelloweye rockfish one of the oldest living rockfishes. Preliminary radiometric age estimates for *C. acrolepis* have been ambiguous compared with the age estimates based on examination of otolith sections. One whole otolith sample was used to calculate the radiometric age estimates. Therefore, variation in ^{226}Ra among the samples could explain the ambiguous results. In order to solve this problem, ^{226}Ra is being measured for each polonium sample. Analysis of *A. pectoralis* is still in its developmental stages, and we will continue to pursue radiometric verification of growth characteristics and longevity.

Cooperating Organizations

Alaska Department of Fish and Game
BioRad Industries
California Department of Fish and Game
Florida Department of Environmental Protection
National Marine Fisheries Service:
  Alaska Fisheries Science Center
  Southwest Fisheries Science Center
Oregon Department of Fish and Wildlife
Scripps Institution of Oceanography
University of Maryland:
  Center for Environmental and Estuarine Studies
  Chesapeake Biological Laboratories

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Spatial Management of the Northern California Red Sea Urchin Fishery

University of California, Davis
R/F-150
1992–94

Louis W. Botsford and James F. Quinn

Both landings data and fishery-independent surveys indicate that the population of the northern California red sea urchin has declined to extremely low levels. Landings have declined from a high of 13.8×10^6 kg in 1988 to 3.3×10^6 kg in 1993. Fishery-independent surveys by the California Department of Fish and Game indicate abundance has declined to about one-third of its former value, and catch-per-unit-effort in the fishery has declined from 311 kg/hr to 114 kg/hr. This study was designed to formulate better ways of assessing the population of these sea urchins, and to develop alternative management strategies involving spatial closures, which might prevent further decline.

Management schemes involving spatial closures provide an effective way of solving one of the most difficult problems in fisheries management, limitation of effort. Two different approaches are used. One, which we began evaluating in our previous Sea Grant project (R/F-136), involves rotating spatial harvest (Botsford et al., 1993). We began the work reported here by evaluating the other, permanent refuges from harvest (Quinn et al., 1993).

Spatial management schemes are particularly appropriate for species with planktonic larvae and Allee effects in reproduction or recruitment. The red sea urchin has dispersing larvae and two potential Allee effects in recruitment. One is the decline in fertilization efficiency at lower densities in broadcast spawners (Levitan, 1991; Levitan et al., 1992). Another is the decline in protection of new recruits under the spine canopies of adults at low densities (Tegner and Dayton, 1977). Both of these make red sea urchins particularly susceptible to overfishing.

One of the key considerations in formulating a scheme involving harvest refugia is the spacing between refugia. Spacing refugia too close together reduces harvest more than is needed, and spacing them too far from one another means that some areas will not be reached by adequate numbers of dispersing propagules. To evaluate this issue, we formulated an age-structured model based on the biology of the red sea urchin. The model consisted of a number of subpopulations distributed along a coastline, with both postdispersal Allee effects (i.e., the spine canopy refuge) and a fertilization Allee effect (Figure 1). Propagules were dispersed along the coast each year over three populations on each side of the propagules' origin.

The effect of reserve spacing on catch and abundance was such that for reserves too far apart, high harvest rates were able to drive all populations to extinction, whereas for spacing below a certain value (three subpopulations in this case), populations persisted despite high harvest rates (Figure 2). Landings at high harvest rates increased as reserve spacing declined. Results with this model also showed that the speed of decline was less with closely spaced refuges, implying that an effective refuge policy would result in more time to adjust policy if populations began to decline. We also evaluated the sensitivity of our

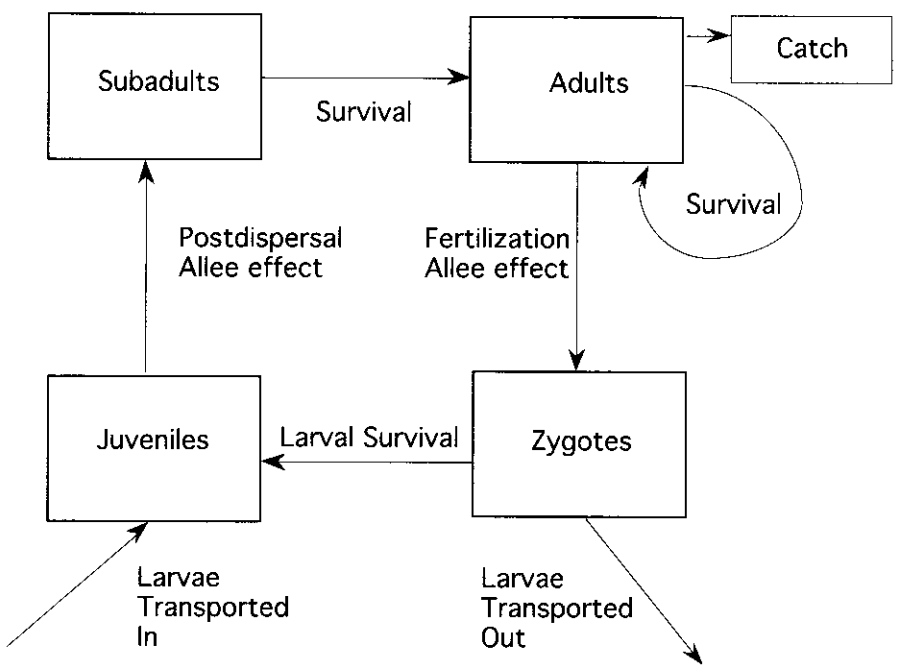

Figure 1. Schematic of model of spatial management of northern California red sea urchins with pre- and postsettlement Allee effects, harvest of adults, and transport of larvae between sites. The model is run with a series of interconnected sites that are designated as reserve (with no harvest) or open (with harvest). The spacing between and size of reserves can be altered to investigate the effects on persistence and harvest.

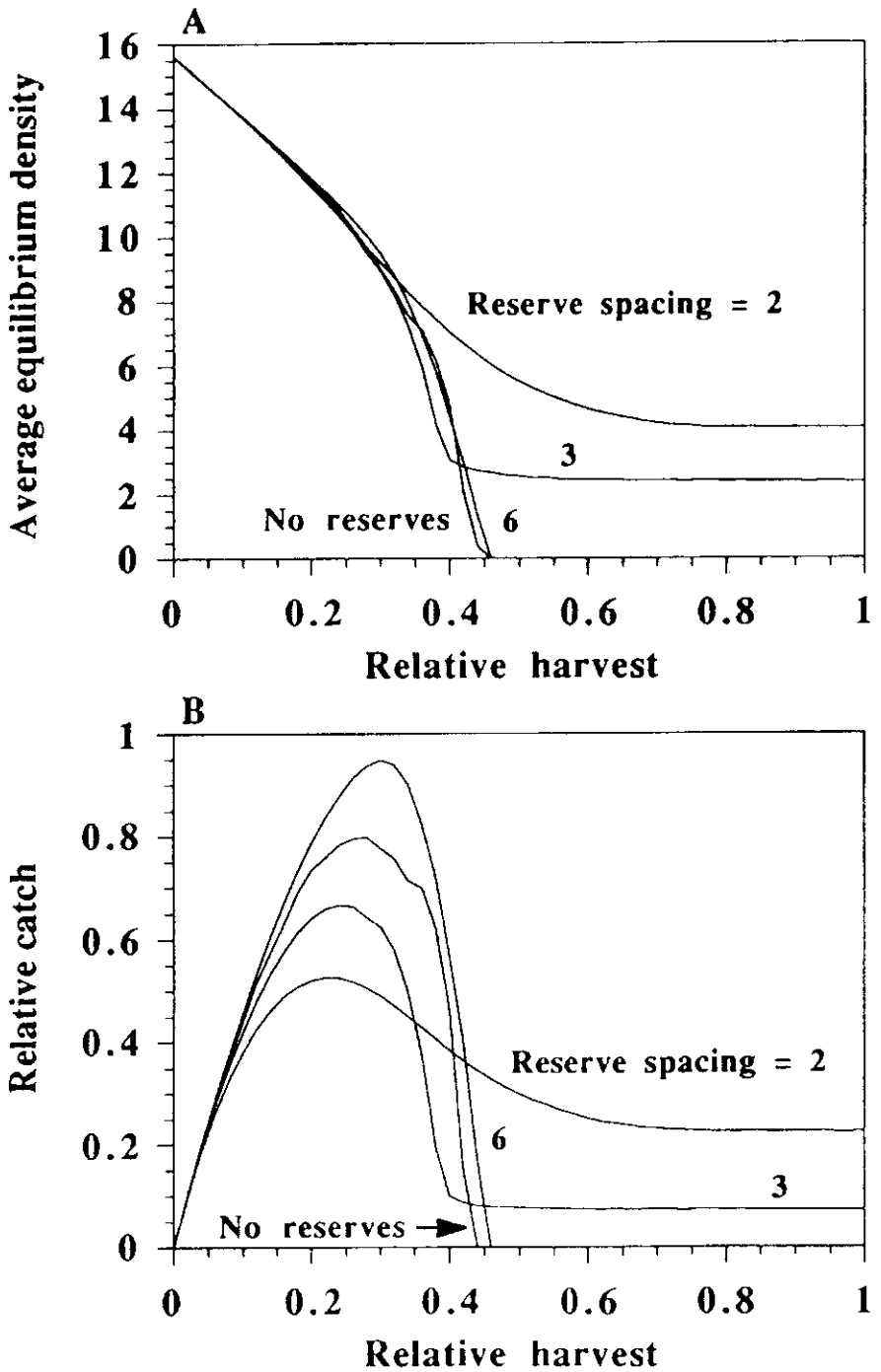

Figure 2. Equilibrium values of population density and catch for the urchin metapopulation model of northern California red sea urchins managed by using harvest refugia for various harvest levels and a range of reserve spacing. Note that population density goes to zero at relative harvests greater than 0.4 with reserve spacings of 6 or greater; populations where harvest refugia are spaced by 2 and 3 population units persist despite high harvest rates.

results to assumed parameter values, such as the parameter describing the decline in fertilization with density, the mortality rate of juveniles beneath the spine canopy, the fraction of larvae dispersing, and the distance dispersed.

Because these results showed that dispersal distances relative to refuge spacing were important in spatial management and because of other similar results for more complex dispersal patterns (Botsford et al.,1994b), we continued our attempts to determine empirically the spatial and temporal variability in urchin dispersal. In the last summer of our previous project (R/F-136), we had monitored settlement of invertebrates by using brush collectors and physical oceanographic conditions near the Bodega Marine Laboratory. One of the first tasks in the project reported here was to analyze settlement and physical data to determine how physical oceanographic conditions influenced recruitment and dispersal along the coast. Because we found an increase in crab settlement on our brush collectors during periods of warmer water when the upwelling winds relaxed, we proposed that a coastally trapped, warm current flowed north from the lee side of Point Reyes, carrying with it crab larvae, which then settled along the coast (Wing et al., 1995a; Send et al., 1987; Figure 3). Settlement of urchins also seemed to be related to this current, but there were far fewer settlement episodes, too few to establish the same degree of predictability as for crabs.

In the following summer, we expanded our sampling to include other points along the coast, to better define the alongshore spatiotemporal variability in invertebrate dispersal and settlement patterns. Once again there was a clear relationship between crab settlement and upwelling relaxation, especially for *Cancer* species (Wing et al., 1995b). Settlement was higher and more constant at the two southernmost points than at the other points (Figure 4). Settlement at the points north of Point Reyes occurred only when upwelling winds relaxed and warm water flowed

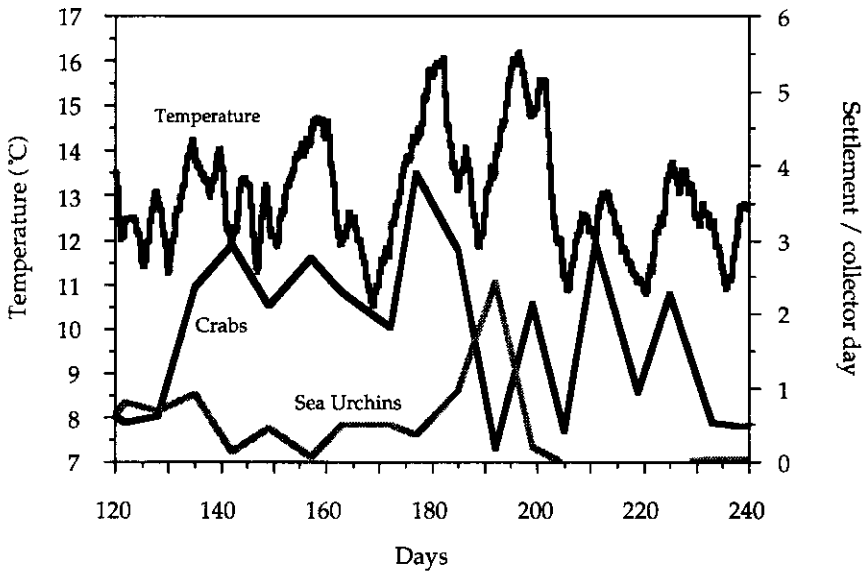

Figure 3. Comparison of settlement of sea urchins and crabs to ocean temperature at Bodega Marine Laboratory in 1993. Graph shows how crab settlement increases with the northward, onshore flow of warmer water during periods of upwelling relaxation (i.e., low or no upwelling).

northward from Point Reyes to Bodega Marine Laboratory and Salt Point. Crab settlement north of Point Reyes was strongly correlated with temperature, whereas settlement at Point Reyes and south was only moderately correlated. Sea urchin settlement was even less than in the previous year, occurring in one temporal pulse only at the tip of Point Reyes.

It is difficult to draw firm conclusions about the dispersal patterns of red sea urchins from these results. However, the results do indicate that settlement is not simply a local process, with larvae settling at the larval point of origin. The findings also show that considerable alongshore transport can occur, in both directions. In addition, they suggest that the spatial scales of dispersal may be limited to the lengths of embayments (e.g., Point Reyes to Point Arena, Point Arena to Cape Mendocino). Similar settlement samples taken by the California Department of Fish and Game in the Point Arena–Cape Mendocino embayment are not correlated with our samples over these 2 years.

One of the benefits of spatial management is that it allows managers to take advantage of spatial variability in productivity. For example, in a scheme with rotating spatial closures, one might harvest more productive areas more often. To determine spatial differences in productivity, we began developing a method for estimating growth and mortality rates from size distribution and increment data. This method originated from work done earlier (R/F-136), in which we evaluated how growth and mortality rates shaped size distributions (Botsford et al., 1994a) and began developing an estimation method based on those observations.

In our method, estimates of growth and mortality are based on size distributions without age modes and size increment data. For reasonable sample sizes (i.e., several hundred size measurements to form the size distribution, and 50 size increments), biases and standard errors of estimated parameters are less than 10%. The parameters typically estimated are L_∞, the maximum size in a von Bertalanffy growth model; the variability in L_∞; k, the rate parameter in a von Bertalanffy growth model; and z, total mortality rate. We have evaluated sensitivity of the estimates to sample size, bin size, measurement error, variability in recruitment, and type of size distribution (mortality dominated, growth dominated, bimodal). The method performs well over a wide range of these parameters.

We will be using this method on size distribution data collected at a variety of locations and two different depths. The locations include both unharvested and harvested areas so that we can use data from the unharvested areas to determine growth and natural mortality rates and then use these values in conjunction with data from harvested areas to estimate fishing mortality rate. To obtain size increment data, we have personal integrated transponder (PIT)-tagged individual urchins in protected locations. PIT-tagging in one location has shown that we can recover approximately one-third of our tags. We have also completed a laboratory growth study at various feeding levels to give us an idea of the range of growth rates to be expected.

After estimating population parameters in a variety of locations, we plan to return to modeling studies to more fully evaluate the two types of spatial harvest policies we have addressed, harvest refugia and rotating spatial harvests.

Cooperating Organizations

California Department of Fish and Game
Gulf of the Farallones National Marine Sanctuary
Scripps Institution of Oceanography

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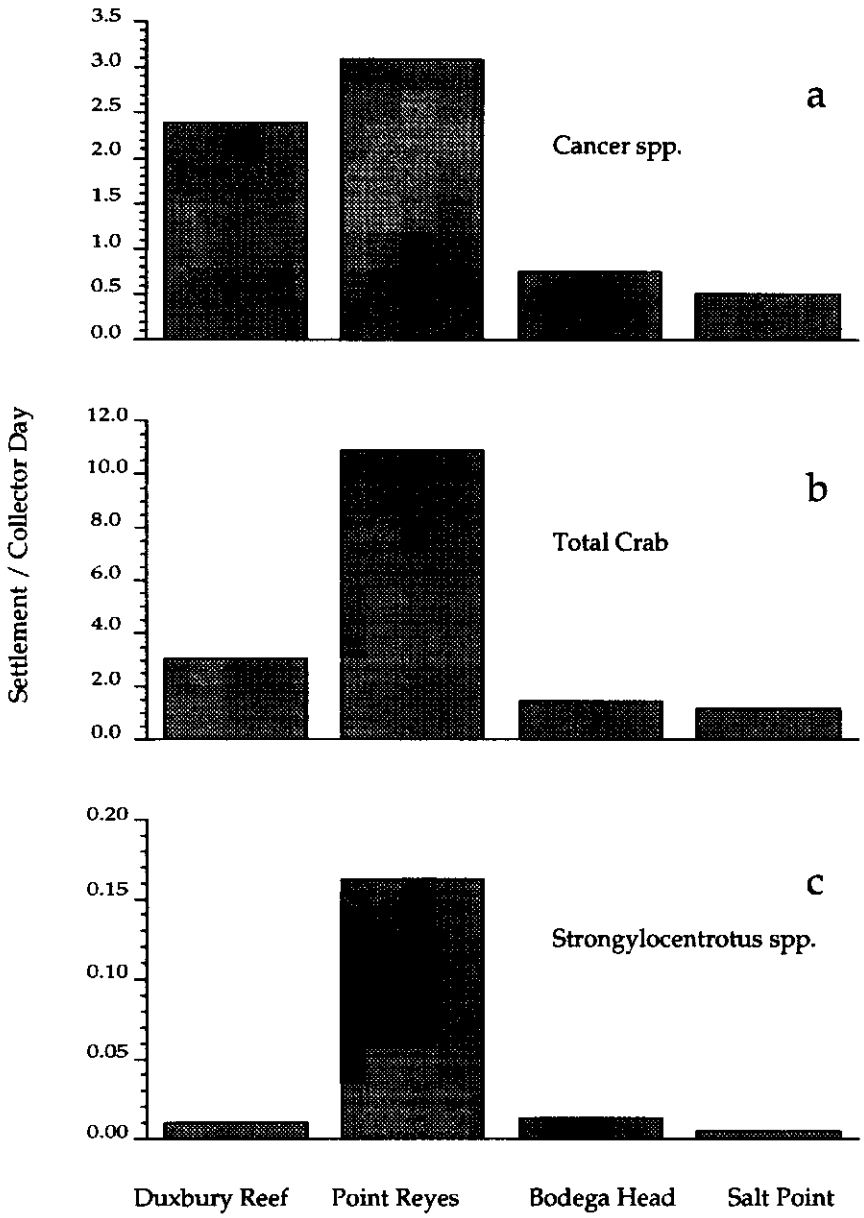

Figure 4. Relative settlement of *Cancer* crabs, total crabs, and sea urchins at four points along the northern California coast through the spring and summer of 1994 reflects the high constant settlement at Point Reyes and to the south and the lower annual total settlement to the north of Point Reyes, which occurs only during upwelling.

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Presentations

Botsford, L.W. Spatial management of a benthic invertebrate, the red sea urchin, *Strongylocentrotus franciscanus*. Conference on Fisheries Management, Anchorage, Alaska, October 1992.

Botsford, L.W. Size structure and recruitment in the northern California red sea urchin fishery. California Cooperative Oceanic Fisheries Investigation (Cal COFI) meeting, Asilomar, California, November 1992.

Botsford, L.W. Harvest of the red sea urchin in northern California: A renewable resource? Presented at a symposium on The Crisis in Invertebrate Conservation, at the annual meeting of the American Society of Zoologists, Vancouver, B.C., December 1992.

Botsford, L.W. Physical oceanographic influences on invertebrate recruitment. EPOC meeting, Fallen Leaf Lake, California, September 1993.

Wing, S.R. Settlement of crab and urchin larvae in the presence of intermittent upwelling. Poster presented at the meeting of the Oceanography Society, Seattle, February 1993; California Cooperative Oceanic Fisheries Investigation (Cal COFI) meeting,, Lake Arrowhead Conference Center, November 1993.

Wing, S.R. Spatial variability in settlement of benthic invertebrates in a northern California upwelling system. Seminar presented at the Bodega Marine Laboratory, California, September 1993; Ocean Sciences meeting, San Diego, February 1994; California Cooperative Oceanic Fisheries Investigation (Cal COFI) meeting, Fallen Leaf Lake, California, October 1994.

New Marine Products

Pharmacological Studies of Inflammatory Processes

University of California, Santa Barbara
R/MP-52
1993–94

Robert S. Jacobs

Developing New Models

Two new models expected to yield new anti-inflammatory drugs have been investigated over the past 2 years. Algae were collected at the Florida Keys Marine Laboratory. Adult horseshoe crabs were obtained from both the Marine Biological Laboratory at Woods Hole and Gulf Specimen in Florida. Juvenile horseshoe crabs were collected at a special habitat on the Indian River in Florida.

In the *Limulus* studies we have demonstrated the presence of an eicosanoid-mediated inflammatory response in *L. polyphemus* by providing evidence of (1) substrate availability and (2) eicosanoid biosynthetic activity. We have determined that the cellular fatty acids in *Limulus* amebocytes contain the eicosanoid precursors linoleic acid, linolenic acid, dihomo-γ-linolenic acid, eicosatetraenoic acid, and eicosapentaenoic acid. The last two fatty acids, arachidonic acid and eicosapentaenoic acid were present in high percentages: 19% and 7%, respectively. Biosynthetic activity was confirmed when whole cells were incubated with exogenous arachidonic acid for 15, 30, 45, 60, and 90 min. Eicosanoid metabolites were purified from the media by using filters and analyzed by thin-layer chromatography. Media collected before and 15 min after addition of arachidonic acid contained only the acid. Media collected after addition of arachidonic acid (30, 45, 60, and 90 min) contained several eicosanoid metabolites in addition to the acid.

Further biosynthesis studies with whole cells incubated with arachidonic acid, the calcium ionophore A23187, or the acid and the ionophore also showed production of eicosanoid metabolite. The metabolites were purified by using filters and analyzed by reverse-phase high-pressure liquid chromatography. The chromatographs indicated that the metabolites included hydroxyeicosatetraenoic acids. In similar assays with radiolabeled arachidonic acid, analysis of the purified metabolites qualitatively indicated the presence of 12-hydroxy-5,8,10-heptadecatrienoic acid, a nonenzymatic cleavage product of prostaglandin H_2, and low levels of prostaglandins in addition to the hydroxyeicosatetraenoic acids. Gas chromatography profiles of methylated metabolites gave profiles similar to those of methylated hydroxyeicosatetraenoic acid standards.

In the algal studies, preliminary studies indicate that *Anadyomene stellata* is capable of producing specific polyunsaturated fatty acids (PUFAs) from palmitoleic 6,9,12,15-octadecatetraenoic, arachidonic, eicosapentaenoic, *cis*-7,10,13,16-docosatetraenoic, and *cis*-4,7,10,13,16,19-docosahexaenoic acids. The metabolites (PUFAs) have unique structures, some of which have not been previously reported in the literature (Fig. 1). Analyses of algal preparations before and after incubation with specific substrates were used to identify and determine the structures of PUFAs. Unique compounds containing conjugated tetraene structures were present in untreated algae preparations, and increased levels of these compounds were found after incubations with specific precursors. Five distinct PUFAs have been identified. One of these five is a previously unreported 22-carbon PUFA with seven double bonds, four of which are conjugated. The presence of these five compounds suggests the existence of an enzymatic cascade that plays a significant role in fatty acid oxidation in *A. stellata*. Further studies with chloroplasts isolated directly from this alga have shown that this enzymatic activity is localized within these organelles.

New Compounds

We have begun the study of two new anti-inflammatory compounds to determine their site and mechanism of action. The first compound, topsentin, is a potent phospholipase A_2 inhibitor with a unique bis-indole structure. This compound is currently under evaluation by Osteoarthritis Sciences, Inc. Our studies indicate that it represents a unique structural species of a phospholipase A_2 inhibitor that probably inhibits the enzyme by binding to a site and through a chemical mechanism that is different from that of manoalide. This conclusion is based on structural considerations only. Topsentin is also a potent typical anti-inflammatory drug that is being studied for its systemic activity.

The second compound is scytonemin, another unique nitrogen-containing anti-inflammatory drug. Our studies of this compound are only preliminary. We have observed inhibition of inflammation after local and systemic administration of scytonemin. This new compound will be studied extensively during this next year.

Follow-up of Active Compounds

Manoalide continues to be an important standard widely used in basic research studies. Our work currently is complete with this material.

Pseudopterosin A methyl ether (methapterosin) is now the number one candidate for development for a number of inflammatory conditions. We have finished preliminary work that shows that it does not block degranulation in mast cells but does block degranulation in neutrophils. Its cellular mode of action remains a mystery, because it also does not

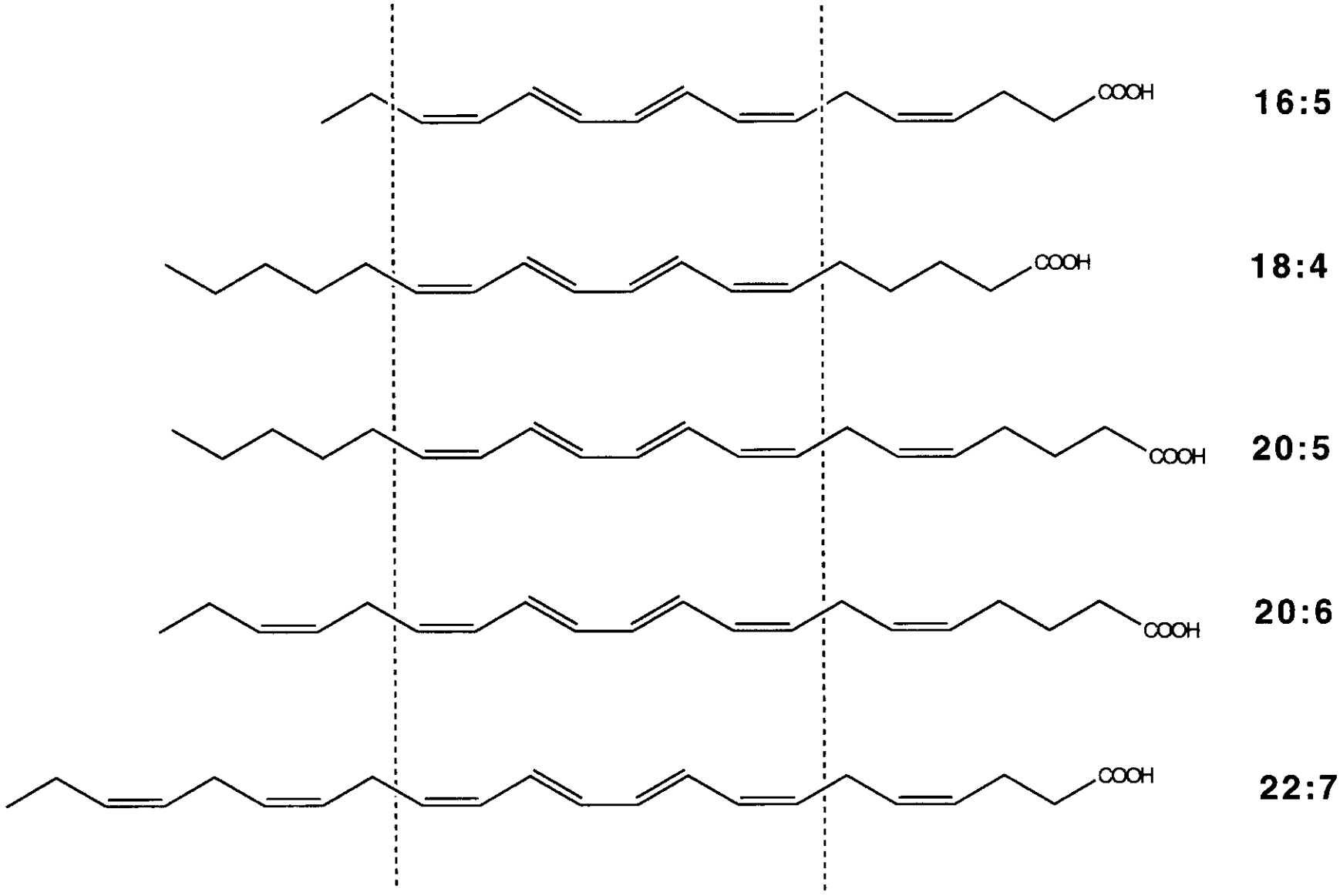

Figure 1. Conjugated tetraenes from *Anadyomene stellata*

affect release of prostaglandins from macrophages. We plan to continue research on its mechanism of action.

Multidrug Resistance

We have completed our study of the effects of marine natural products in multidrug-resistant cancer cells. The publications are being prepared, and Allen Willams is writing his thesis on this subject.

Special Note

The marketing of one of our marine natural products has begun. A mixture of natural products has been incorporated into a cream and used for its general anti-inflammatory properties. This is the first compound from our research program to reach this stage of development, although this was an unexpected application.

The benefits of this application of our basic research have been to increase employment, create a special activity in the fishing industry for workers in the Bahamas, and increase market activity in skin care products.

Cooperating Organizations

Florida Keys Marine Laboratory, Long Key, Florida

OsteoArthritis Sciences, Inc., Boston, Masschusetts

Smithsonian Marine Laboratory, Linkport, Florida

Presentations

Williams, A.B. A cyclic octapeptide isolated from *Lissaclinum patella* reverses multidrug resistance in a human leukemic cell line. Poster presented at the 84th annual meeting of the American Association for Cancer Research, Orlando, Florida, May 1993.

Williams, A.B. Use of a cyclic peptide isolated from *Lissaclinum patella* in reversal of multidrug resistance in a human leukemic cell line. Poster presented at the Gordon Research Conference, 10th annual Marine Natural Products Meeting, Ventura, California, February 1994.

Mikhailova, M. Eicosanoid biosynthesis in the primative green algae *Anadyomene stellata*. Poster presented at the Gordon Research Conference, 10th annual Marine Natural Products Meeting, Ventura, California, February 1994.

MacPherson, J. The Biosynthesis of eicosanoids in the amebocyte *of Limulus polyphemus*. Poster presented at the Gordon Research Conference, 10th annual Marine Natural Products Meeting, Ventura, California, February 1994.

Jacobs, R.S. Topsentin, a unique phospholipase A_2, inhibitor. Presented at the Japan–U.S. Seminar on Bioorganic Marine Chemistry, Numazu, Japan, July 1994.

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Williams, A.B., and R.S. Jacobs. 1993. A marine natural product, patellamide D, reverses multidrug resistance in a human leukemic cell line. *Cancer Lett.* 71:97– 102.

Jacobs, R.S., M. Bober, I. Pinto, A.B. Williams, P.B. Jacobson, and M.S. de Carvahlo. 1993. Pharmacological studies of novel marine metabolites. In D. Attaway, and O. Zaborsky, eds., *Advances in Marine Biotechnology*. Vol. 1: Plenum Press, New York, pp. 77–99.

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Marine Inflammation and Metal-Ion Conjugation

University of California, Santa Barbara
R/MP-53
1992–95

Alison Butler

The objectives of Sea Grant proposal R/MP-53 were (1) to investigate the selectivity of the halogenation reactions catalyzed by vanadium bromoperoxidase (V-BrPO), including the reactivity with pseudohalides such as thiocyanate and cyanide and the reactivity with hydrogen peroxide, and (2) to isolate, purify, and determine the structures of siderophores from marine bacterial isolates and related strains and begin investigations of the metal-binding selectivities of these new siderophores.

Reactions Catalyzed by Vanadium Bromoproxidase

We reported the first evidence that V-BrPO can bind certain organic substrates (i.e., indoles) and that the active brominating moiety under these conditions is not an enzyme-released bromine species (Tschirret-Guth and Butler, 1994). We have followed up on these results with photoaffinity labeling studies to address the specificity of binding of indoles to V-BrPO. We have found that 1,2-dimethylindole reacts specifically with the peroxidase. This part of the project has involved synthesis of the new photoaffinity label [^{3}H]-5-azido-1,2-dimethylindole, photolysis of V-BrPO with [^{3}H]-5-azido-1,2-dimethylindole, and analysis of the proteolytic digests of [^{3}H]-1,2-dimethylindole-labeled V-BrPO. At this point, we have shown that the [^{3}H]-indole-label resides with a single peptide fragment.

Hydrogen peroxide, which is a substrate of V-BrPO, is a noncompetitive inhibitor of V-BrPO. Hydrogen peroxide inhibition increases with increasing pH (Soedjak et al., 1995). The inhibition is reversible under the conditions of the initial steady-state kinetic experiments. Analysis of the inhibition constants versus hydrogen ion concentration indicated that an ionizable group with a pK_a between 6.5 and 7 is involved in the inhibition. Labelling experiments have shown that the oxygen atoms in the dioxygen produced by the V-BrPO–catalyzed bromide-assisted disproportionation of hydrogen peroxide originate from the same molecule of hydrogen peroxide. V-BrPO–catalyzed bromination is an electrophilic (Br^+) rather than a radical ($Br\bullet$) process. The stoichiometry of hydrogen peroxide consumed to monochlorodimedone (MCD) reacted or to oxygen produced is reported. The concentration of hydrogen peroxide also affects the competition of dioxygen formation during MCD bromination; competitive dioxygen formation is strongly enhanced at high pH. Turnover of V-BrPO under conditions of high concentrations of hydrogen peroxide leads to irreversible inactivation at pH 4 and pH 5. Much less inactivation occurs during turnover at long reaction times at higher pH (> pH 6), and the inactivation can be fully reversed by treatment with vanadate.

We have also shown that V-BrPO isolated from marine algae can catalyze the oxidation of the pseudohalide thiocyanate by hydrogen peroxide. Thiocyanate inhibited bromide peroxidation through preferential oxidation of thiocyanate over bromide. Oxidized thiocyanate generated by the V-BrPO/NCS-/H_2O_2 system oxidizes 5-thio-2-nitrobenzoic acid to 5,5-dithio-bis(2-nitrobenzoic acid. V-BrPO catalyzes the thiocyanation of 1,3,5-trimethoxybenzene and 1,2-dimethylindole to 1-thiocyanato-2,4,6-trimethoxybenzene and 1, 2-dimethyl-3-thiocyanato-indole, respectively. Nuclear magnetic resonance studies of the oxidation of KS^{13}CN by hydrogen peroxide catalyzed by V-BrPO showed the formation of several oxidized thiocyanate species, including the putative dithiocyanate ether, which is unstable; hypothiocyanate; thiooxime; and bicarbonate (Walker and Butler, 1996).

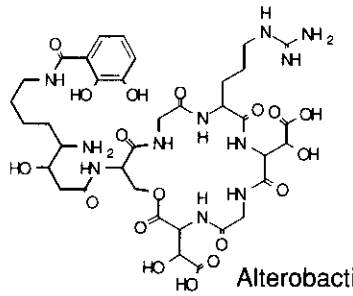
Siderophores

We have determined the structures of alterobactin A and alterobactin B, isolated from the oceanic bacterium *Alteromonas luteoviolacea* (Figure 1). Alterobactin A has an unusual structure with two ß-hydroxyaspartate iron(III)-binding moieties and one catecholate iron(III)-binding moiety. The unique feature of this siderophore is its exceptional iron(III) affinity constant of up to 10^{48}. Alterobactin B forms a 2:1 ligand to metal complex with ferric ion (Reid et al., 1993; Reid, 1994). The actual structure of the complex is unknown, but iron binding appears to involve both catechols. The complex occurs in two different pH-dependent forms (Reid, 1994). Working in collaboration with Professor George Luther, College of Marine Studies, University of Delaware, we estimated the ferric affinity constant of Fe(III)(alterobactin B)$_2$ at pH 6 and 8.2: Log K 37.6 at pH 6 and Log K 43.6 at pH 8.2 (Lewis et al., 1995).

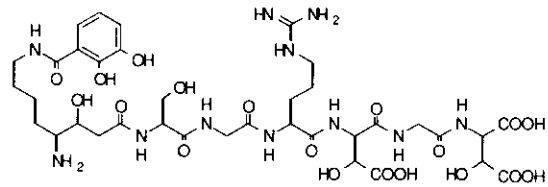
We have isolated and determined the structure of the siderophore putrebactin (Figure 1) produced by the bacterium *Shewanella putrefaciens*. Putrebactin is a cyclic bis hydroxamate siderophore that binds ferric ion in a 3:2 putrebactin: Fe(III) ratio at neutral pH and a ratio of 1:1 at acidic pH. *Shewanella putrefaciens* is important in fish spoilage and pipeline corrosion.

In addition, we have recently determined the structures of a series of four new siderophores from an open-ocean bacterial isolate, DSM40, obtained from Professor Margo Haygood, Scripps Institution

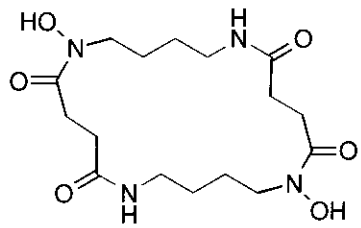
of Oceanography (Figure 2). These structures were determined by electrospray mass spectrometry sequencing for the peptide fraction and by nuclear magnetic resonance for the fatty acid part.



Alterobactin A



Alterobactin B



Putrebactin

Figure 1. Structures of three marine siderophores.

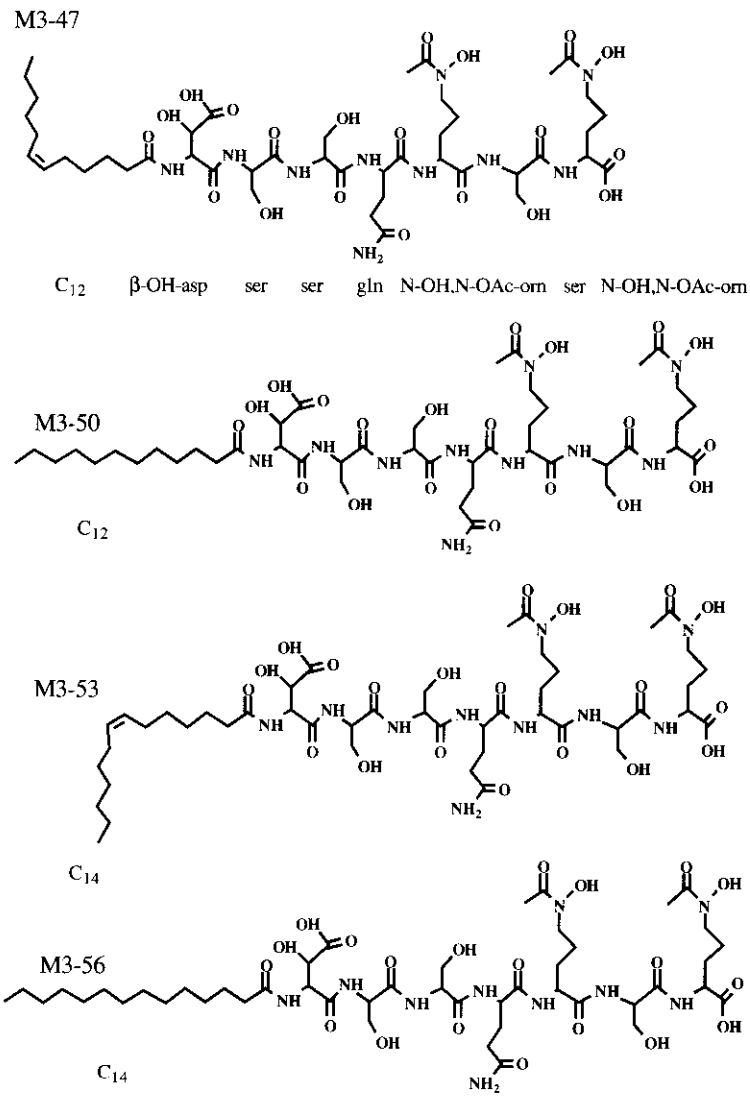


Figure 2. Structures of four siderophores from an open-ocean bacterial isolate.

Cooperating Organizations

Johnson and Johnson Diagnostics, Rochester, New York

OsteoArthritis Sciences, Inc., Cambridge, Massachusetts

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Departments of Chemistry, Harvard University and Massachusetts Institute of Technology, March 1993.

Symposium on Mechanistic Bioinorganic Chemistry, National American Chemical Society meeting, Denver, March–April 1993.

Department of Chemistry, Occidental College, September 1993.

Alterobactins: New siderophores from marine bacteria. Presented at Metals in Biology Gordon Conference, Ventura, California, January 1994.

Iron acquisition in the ocean: Siderophores from oceanic bacteria and metal coordination reactivity. Presented at the American Chemical Society meeting, San Diego, March 1994.

Department of Chemistry, University of California, Santa Barbara, March 1994.

Samsun Medical Research Foundation, Santa Barbara, April 1994.

Mechanisms of iron acquisition by marine microorganisms: Bacterial siderophores. Presented at NSF/ONR workshop on iron speciation and biological availability in seawater, Bermuda Biological Station, April–May 1994.

Vanadium in Biology, 4th Inorganic Biochemistry Summer Workshop, University of Georgia, July–August 1994.

Marine Biotechnology Symposium: Economic Opportunities for California, San Diego, October 1994.

Adventures in discovery: Marine and freshwater systems, 25th Anniversary Celebration Marine Science Institute, University of California, Santa Barbara, October 1994.

Vanadium bromoperoxidase: Enzyme and functional mimetic studies. Presented at the 7th International Conference on Bioinorganic Chemistry, Lübeck, Germany, September 1995.

Biochemical Halogenation. Whistler Center for Carbohydrate Research, Purdue University, September 1995.

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Butler, A. Selectivity of vanadium bromoperoxidase and new siderophores from marine bacteria. Presented at Marine Natural Products, Gordon Conference, February 1994.

Butler, A., M.J. Clague, G.E. Meister, and H.S. Soedjak. Vanadium bromoperoxidase: Enzyme and biomimetic studies. Poster presented at Metals in Biology, Gordon Conference, Ventura, California, January 1995.

Holt, P.D., and A. Butler. Marine siderophores. Poster presented at the 1st conference on Marine Biotechnology: Emerging Economic Opportunities for California, San Diego, October 1994.

Holt, P.D., and A. Butler. Siderophores from marine bacteria: Structural and metal binding characteristics. Poster presented at the 7th International Conference on Bioinorganic Chemistry, Lübeck, Germany, September 1995.

Lewis, B.L., S.W. Taylor, G.W. Luther III, P.D. Holt, A. Butler, S.W. Wilhelm, and C.G. Trick. Voltammetric estimation of iron(III) thermodynamic stability constants for catecholate siderophores isolated from marine bacteria and Cyanobacteria. Poster presented at the American Geological Union meeting, October 1994, and the Chemical Oceanography, Gordon Research Conference, Henniker, New Hampshire, June 1995.

Meister, G.E., and A. Butler. biophysical studies of vanadium bromoperoxidase: probing the vanadium active site. Presented at the 208th National American Chemical Society meeting, Washington DC, August 1994.

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Marine Inflammation Research Program: Anti-Inflammatory Agents from Cultured and Collected Marine Organisms

Scripps Institution of Oceanography
R/MP-54
1992–94

William Fenical

The main goals of the Marine Pharmaceutical Discovery Program were to (1) explore marine plants and animals for new leads in the treatment of inflammatory diseases and related disorders in lipid metabolism and (2) further define existing discoveries, with a major emphasis on the elucidation of novel mechanisms of pharmacological action. Additional goals were to continue to develop the commercial application of the pseudopterosins and to continue two new research collaborations, with Ligand Pharmaceuticals in La Jolla and Osteo-Arthritis Sciences Inc. in Boston. The project included two expeditions to the Bahama Islands as part of our new lead exploration program. We have continued to interact with researchers at Ligand and Osteo-Arthritis Sciences, and we have made two discoveries that have been further evaluated. Two exciting developments, the final commercialization of the pseudopterosins as anti-inflammatory agents in skin care products and preparation for clinical trials of pseudopterosin A methyl ether in late 1994, have been our major developments. Details of these studies follow.

New Drug Discovery Research: Field Research Programs

As part of our collaborative field program to discover new drug leads, we have continued to collaborate with the Jacobs group from the University of California, Santa Barbara, in the operation of the University of Miami's research vessel *Columbus Iselin*. During the summer of 1993, we did onboard collaborative research, which consisted of collecting marine organisms and doing integrated chemistry and biological assays, as part of a 21-day expedition to the Bahama Islands. The program receives support from the National Science Foundation, and the Sea Grant research is highly complementary to its goals. In 1994, we transferred our operation to the research vessel *Seward Johnson*, operated by the Harbor Branch Oceanographic Institution, for a second Bahamas expedition. In this field program, we began to investigate the ascidians from the Caribbean Sea and from La Jolla and surrounding waters. We have discovered several new molecules of interest. In an unidentified Caribbean ascidian, we found a series of radically new aromatic alkaloids, the didemimides A–C (**1–3**, Figure 1). These compounds are undergoing pharmacological study. In another study, we have isolated a novel series of alkaloids from another ascidian, *Didemnum* sp. One of these compounds (**4**) is a potent inhibitor of phospholipase A_2, an enzyme in the inflammatory pathway.

New Agents Through Microbial Fermentation

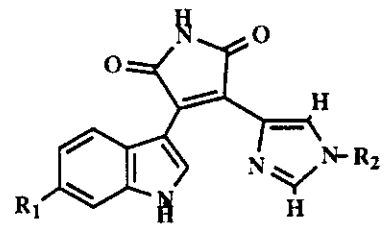
Another aspect of our Sea Grant research has been the development of microbiological, particularly bacterial, resources in marine environments. Because fermentation products from soil bacteria have been the foundation of the modern pharmaceutical industry, we made significant efforts to understand the distribution, variability, and culture requirements of marine gram-negative and gram-positive bacteria. In work with bacteria isolated from sediments of local estuaries, we discovered a novel antibacterial agent (**5**) and isolated at least two novel molecules, a peptide (**6**) and a small acid (**7**).

In conjunction with our fermentation work, we have isolated and defined two potent new anti-inflammatory agents produced by marine bacteria. Marine bacterial strain CNB-382 produces the cyclic peptide cyclomarin A (**8**). This compound has an exceptionally novel structure and is one of the most potent topical anti-inflammatory agents we have discovered. In recent testing, cyclomarin A had significant *in vivo* activity. Two other potent topical ant-inflammatory agents isolated from marine bacteria are lobophorins A and B (**9** and **10**), two macrolides with excellent topical activities. These compounds are related to a known class of antibiotics, the kijianolides, but they have significant differences. Lobophorin A is the amino analogue of lobophorin B, a nitro sugar derivative. In recent testing, lobophorins A and B showed *in vivo* activity. The overall observation of in vivo activity with these two compounds underscores their importance as new leads in the development of anti-inflammatory agents.

Development of Pseudopterosins

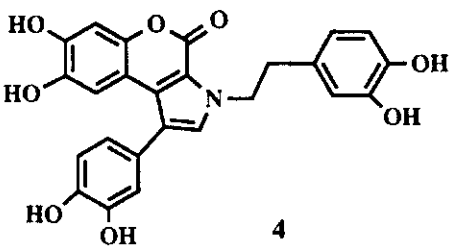
Over the past 2 years, we have continued to interact with the Jacobs group and with several industries to foster the commercial development of the pseudopterosin class of anti-inflammatory agents. Our major goals were to encourage the additional investments required for the agents' development. One major success in commercializing the pseudopterosins is their effective use in skin creams. A subcontractor of a major cosmetics company was awarded an exclusive license by the University of California to market the pseudopterosins as part of a new line of outdoor skin care products. The University has already begun to receive royalty payments from this license.

As part of our development of the pseudopterosins for medicinal applications, we have concentrated on the major clinical candidate,



	R_1	R_2
A:	H	H
B:	H	CH_3
C:	Br	CH_3

1-3



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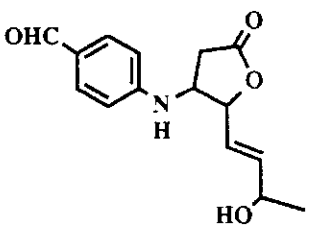

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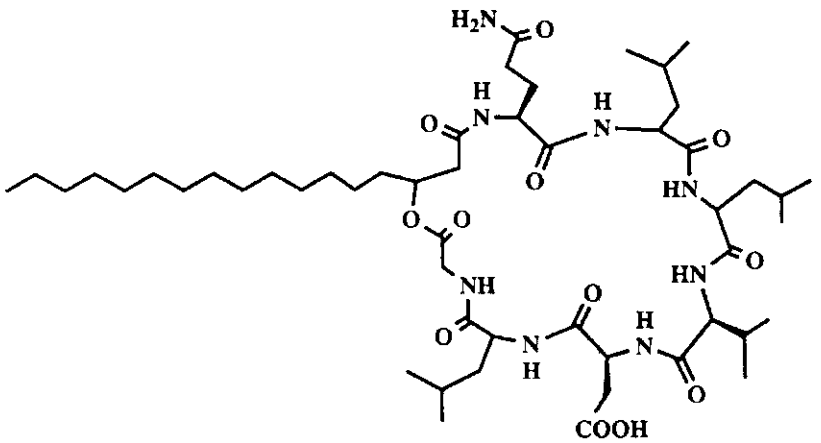

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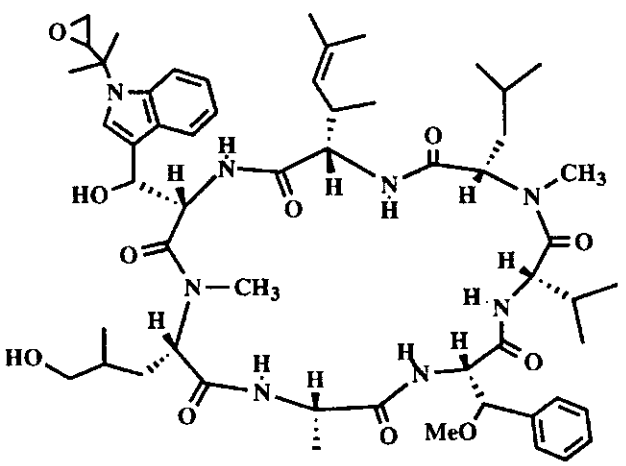
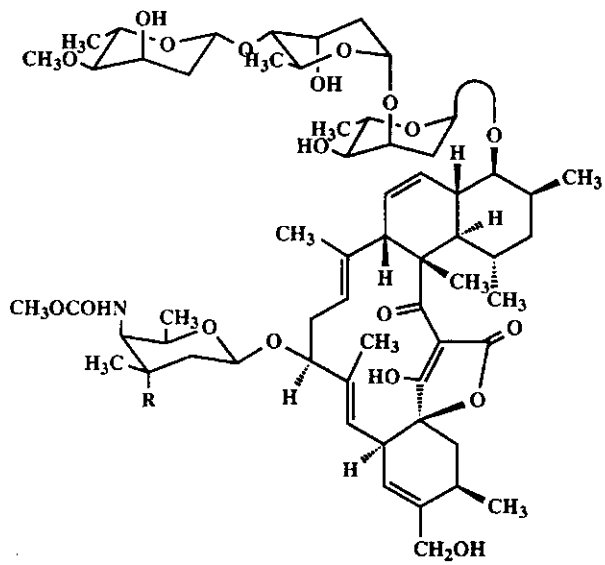
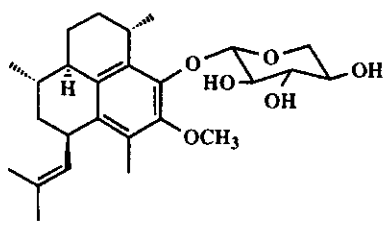


Figure 1. New bioactive compounds from marine organisms.



9, R = NH_2

10, R = NO_2



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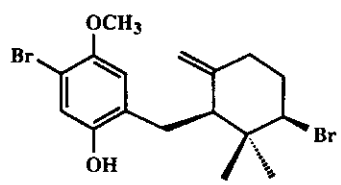

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Figure 1. (continued)

pseudopterosin A methyl ether (**11**), now known as methylpterosin, which has been licensed by OsteoArthritis Sciences Inc. This compound is superior to the natural compounds, and we have selected it for our clinical study. In 1994 OsteoArthritis Sciences began the large-scale isolation process. Surprisingly, only about 20 kg of *Pseudopterogorgia elisabethae* was required for the entire evaluation. We have provided complete guidance for this activity, including the information for extraction, purification, and conversion of pseudopterosin A to the methyl ether.

Discovery of a Novel Progesterone Analogue

As mentioned, we have dedicated considerable efforts toward a new interaction with Ligand Pharmaceuticals in La Jolla. The basis of Ligand's approach to drug discovery is the application of intracellular receptors in drug screening.These receptors are the natural binding sites for drugs and natural hormones. By cloning these receptors and establishing novel bioassay methods, Ligand can screen for analogues of existing drugs and hormones that are as effective as but much safer than the existing compounds. An example is the vitamin D receptor. In principle, 1, 25-dihydroxyvitamin D would be an effective drug to regulate calcium metabolism. Unfortunately, the vitamin has serious side effects that completely restrict its use in this regard. Our interactive program seeks to detect new molecules that bind to the vitamin D receptor and other receptors but lack the serious toxic effects often observed.

Our main approach has been to screen many compounds produced by marine bacteria. We have isolated and identified at least three new classes of selective binding agents. These observations are the first to prove that "mimics" of the natural agents can be isolated and possibly developed as new drugs. We have isolated a highly selective antagonist of the progesterone receptor that has been patented by Ligand and the University of California. The molecule discovered is a

bromoterpenoid (**12**) isolated from the green alga *Cymopolia barbata*. It is a mechanistically novel substance, and it has been the subject of a significant synthesis effort at Ligand. Analogues have been prepared and patented. The compound has been obtained in larger quantities and is now being evaluated in *in vivo* tests.

Cooperating Organizations

Ligand Pharmaceuticals, La Jolla, California

OsteoArthritis Sciences, Inc., Boston, Massachusetts

Publications

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New metabolites from Indian Ocean marine invertebrates. Invited Lecture, Asian Symposium on Medicinal Plants and Spices, Manila, February 1992.

Marine bacteria: A new biomedical resource. Invited Lecture, annual meeting of the American Association for the Advancement of Science, Chicago, February 1992; invited lecture, Upjohn Pharmaceuticals, Kalamazoo, Michigan, June 1992; annual meeting of the Society of Industrial Microbiology, San Diego, August 1992; invited lecture, Monsanto Company, St. Louis, October 1992.

Marine Bacteria: Symbiosis and the production of bioactive metabolites. Invited lecture, Gordon Research Conference on Marine Natural Products, Ventura, California, February 1992.

The ocean: A sea of chemicals. Nevada Regional Section of the American Chemical Society, University of Nevada, Reno, May 1992.

Bacterial adaptations in the ocean: A guide to a new biomedical resource. Southern California Section of the American Society for Microbiology, October 1992.

Antibiotics from the sea: Cures for the next decade. Invited lecture, 30th annual New Horizons in Science Meeting, Council for the Advancement of Science Writing, Inc., La Jolla, November 1992.

Marine bacteria as a new hemotherapeutic resource. Plenary lecture, Annual meeting of the Pharmaceutical Society of Japan, Osaka, March 1993; Plenary lecture, 3rd International Meeting of the Society of Industrial Microbiology, Rohnert Park, California, April 1993.

The oceans: A medical frontier. Institute for Continual Learning, University of California, San Diego, May 1993; The Covey, Natural History Museum, San Diego, September 1993.

New frontiers in marine natural products chemistry. Plenary lecture, 18th Mardi Gras Symposium, Louisiana State University, New Orleans, February 1994.

Drugs from the sea. Oceanography lecture series, Solana Beach Commission for Parks and Recreation, Solana Beach, California, March 1994.

Marine chemosensory processes for communication and defense. Plenary lecture, 16th Annual meeting of the Association for Chemoreception Sciences, Sarasota, Florida, April 1994.

The ocean, A unique chemical environment. Departmental lecture, California State University, Northridge, September 1994.

Marine Inflammation Research Program: Anti-Inflammatory Agents from Marine Invertebrates

Scripps Institution of Oceanography, UCSD
R/MP-55
1992–94

D. John Faulkner

During the past 2 years, 35 new marine natural products were submitted by Robert S. Jacobs, University of California, Santa Barbara for pharmacological evaluation. Seven of these compounds showed significant anti-inflammatory activity in the mouse ear assay, five inhibited bee venom phospholipase A_2, and two inhibited cell division in fertilized sea urchin eggs. Further studies of these compounds are in progress. Details of the biological screening data are reported in the Annual Report of Screening Activities for the years 1992–1993 and 1993–1994 prepared by Krista Grace and Professor Jacobs.

A second collaboration with Vivek Malhotra of the Biology Department, University of California, San Diego, has resulted in the discovery that the sponge metabolite ilimaquinone (**Figure 1, 1**) causes complete vesiculation of Golgi membranes and inhibition of protein transport (Takizawa et al., 1993). Additional studies of the effects of other natural products from sponges on Golgi membranes, microtubule assembly, and protein transport will be published soon. We have designed an assay to screen crude extracts for their effects on these basic cellular mechanisms. In the first batch of 60 extracts, 4 extracts showed useful activity in the assay (this does not include those extracts that simply kill the cells). This project has reached the stage at which separate funding is required for more advanced studies.

Earlier results from our collaborative research projects have been used to select compounds to send to OsteoArthritis Sciences Inc. for screening in proprietary assays. We have had some initial success, which has resulted in discovery of a lead compound in the chondrocyte matrix breakdown assay. The lead compound has been reisolated from a second sponge specimen, and sufficient material has been obtained to proceed with preclinical testing. A second group of sponge metabolites are also active in the chondrocyte matrix breakdown assay, but these are less attractive as potential drugs.

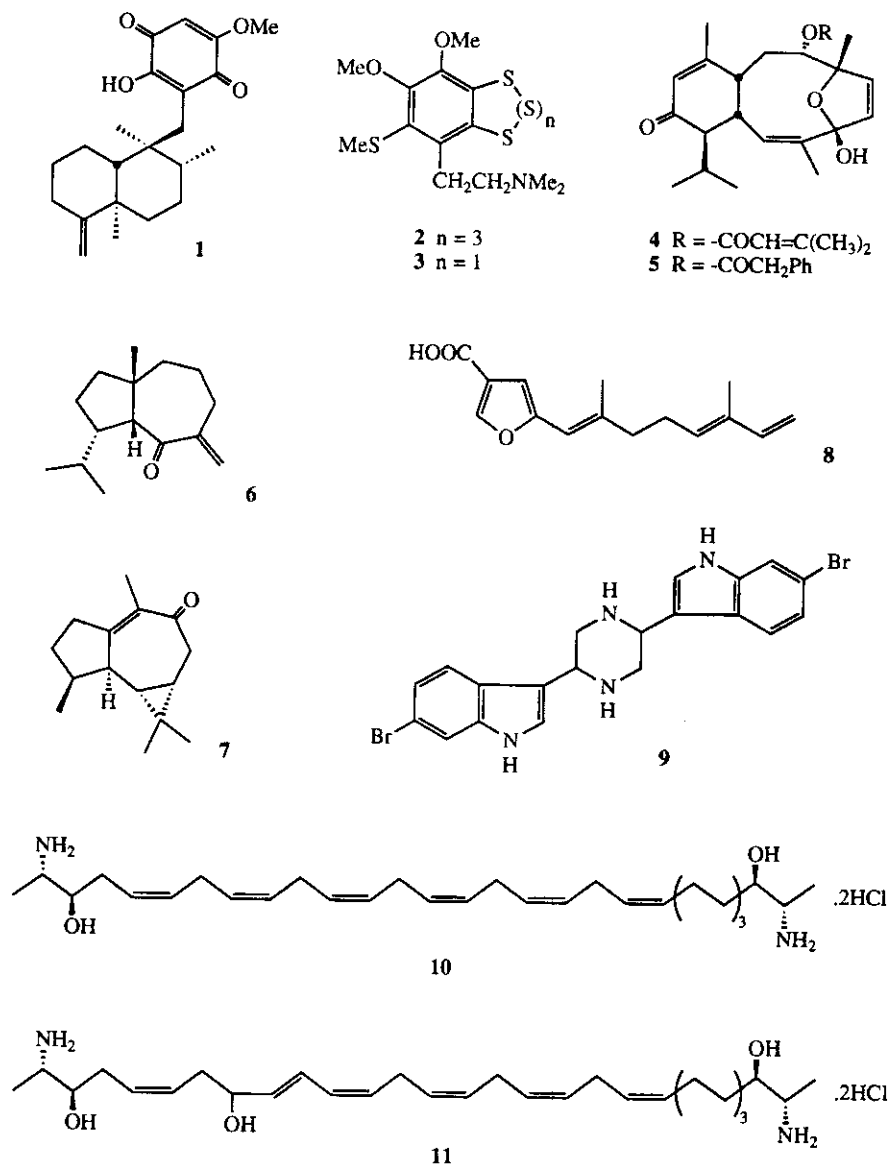
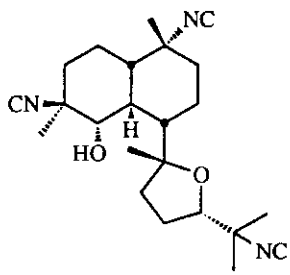


Figure 1. Structures of recently isolated natural products from marine invertebrates. See text for types and sources.

In collaboration with colleagues at SmithKline Beecham, we have described a series of new pentathiepins and trithianes that inhibit protein kinase C, which were obtained from marine tunicates: the compounds isolated at Scripps Institution of Oceanography were the pentathiepin (**2**) and the trithiane (**3**) from a *Lissoclinum* sp. (Compagnone et al., 1994). Collaborations with the pharmaceutical



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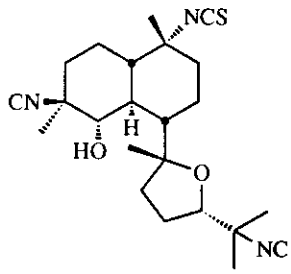

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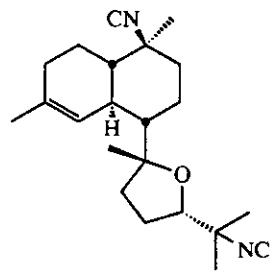

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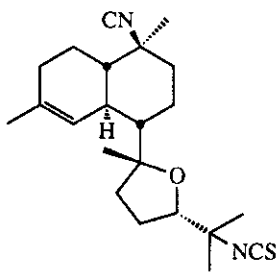

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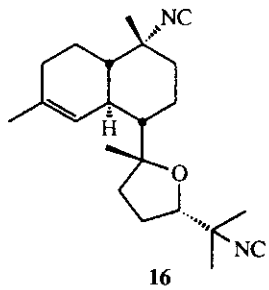

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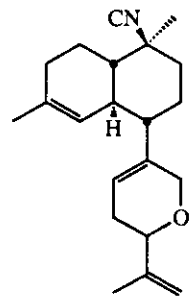

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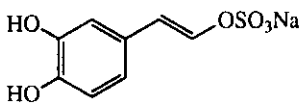

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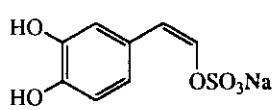

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industry are important to our Sea Grant research program, because they can extend our basic research on anti-inflammatory compounds from marine invertebrates while providing matching funds.

The results of our initial screening of the extracts of South African marine invertebrates for cytotoxic effects and antimicrobial activity were disappointing. We were therefore pleased to discover that valdivones A (**4**) and B (**5**), from the South African soft coral *Alcyonium valdivae*, have significant antiinflammatory activity (Lin et al., 1993). Collection of more samples of this soft coral from the Transkei will not be possible until next year. Mike Davies-Coleman, our collaborator at Rhodes University, has examined specimens of *Alcyonium*, including *A. valdivae*, from around Port Elizabeth, but these specimens did not contain the same chemicals. We do not usually submit compounds from nudibranchs for screening because they are difficult to obtain in large quantities. However, the sesquiterpenes from the South African nudibranch *Leminda millecra* (Pika and Faulkner, 1994) were screened by Dr. Jacobs, and millecrones A (**6**) and B (**7**) were found to be inhibitors of phospholipase A_2. Dr. Davies-Coleman has been asked if he can supply more nudibranchs or the dietary soft coral from which the nudibranchs were probably obtained.

For certain simple marine natural products, chemical synthesis may provide an advantage over isolation from natural sources, particularly when larger quantities of material are required for evaluation. We have completed the syntheses of the furanoic acid (**8**) from soft corals of the genus *Sinularia*. The furanoic acid was shown to have useful anti-inflammatory activity (Grace et al., 1994). Sea Grant trainee Ted Evans is working on synthesis of 2,5-bis-(6′-bromo-3′-indolyl)-piperazine (**9**) from the Mexican tunicate *Didemnum candidum* (Fahy et al., 1991).

Other studies reported in the last year include the isolation and structural elucidation of leucettamols A (**10**) and B (**11**), which are antimicrobial agents from the calcareous sponge *Leucetta microraphis* (Kong and Faulkner, 1993), the isolation of six new diterpene isonitriles (**12–17**) from the sponge *Acanthella cavernosa* (Trimurtulu and Faulkner, 1994), the isolation of two enol sulfates (**18** and **19**) from a two-sponge association (Cerda-García-Rojas et al., 1994), and two collaborative studies of metal binding by marine natural products (James et al., 1993; Reid et al., 1993). The metal-binding studies are important not only because they may shed light on the function of marine natural products in the natural environment but also because they may inhibit enzymes that contain metals at the active sight.

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Ocean Engineering and Instrumentation

A Knowledge-Based Approach for Assessing the Integrity of Offshore Platforms Subjected to Seismic Loading

University of California, Berkeley
R/OE-19
1992–94

C. William Ibbs and Robert G. Bea

This research project produced an information management system (IMS) for the systematic structural screening of offshore petroleum drilling, production, and quarters facilities in the state and federal waters of offshore California. The IMS also stores and manages much of the information required by regulatory and industry for further structural requalification of such platforms.

Platform Screening

The screening portion (Figure 1) of the IMS uses a first-level (level 1) process of assessment to rapidly evaluate how remedial work on structures might improve their safety and reliability. This process includes procedures for structural analysis, consequence evaluation, and risk assessment that must be used together to evaluate a structure's integrity. The IMS has provisions for the later incorporation of second-, third-, and fourth-level (levels 2, 3, and 4, respectively) structural assessments. The higher the assessment level is, the more expensive, time-consuming, and accurate the analysis. Thus, an efficient level 1 analysis inexpensively indicates where more effort should be devoted.

As incorporated, the level 1 structural assessment uses an expression for reserve strength ratio (RSR, a measure of structural worthiness) as reported in Bea and Craig (1993):

$$RSR = \frac{R_1 \cdot R_2 \cdot R_3 \cdot R_4 \cdot R_5}{S_1 \quad S_2 \quad S_3 \quad S_4}$$

R_1, R_2, R_3, R_4, and R_5 are subjective evaluations of capacity effects (structure and foundation design and construction criteria, structure condition, structure and foundation modification; structure and foundation configuration, and loading-capacity effects factor F_v, respectively). S_1, S_2, S_3, and S_4 are subjective evaluations of loading effects (storm loading design criteria, deck elevation, loading modifications, and operating and gravity loading modifications, respectively).

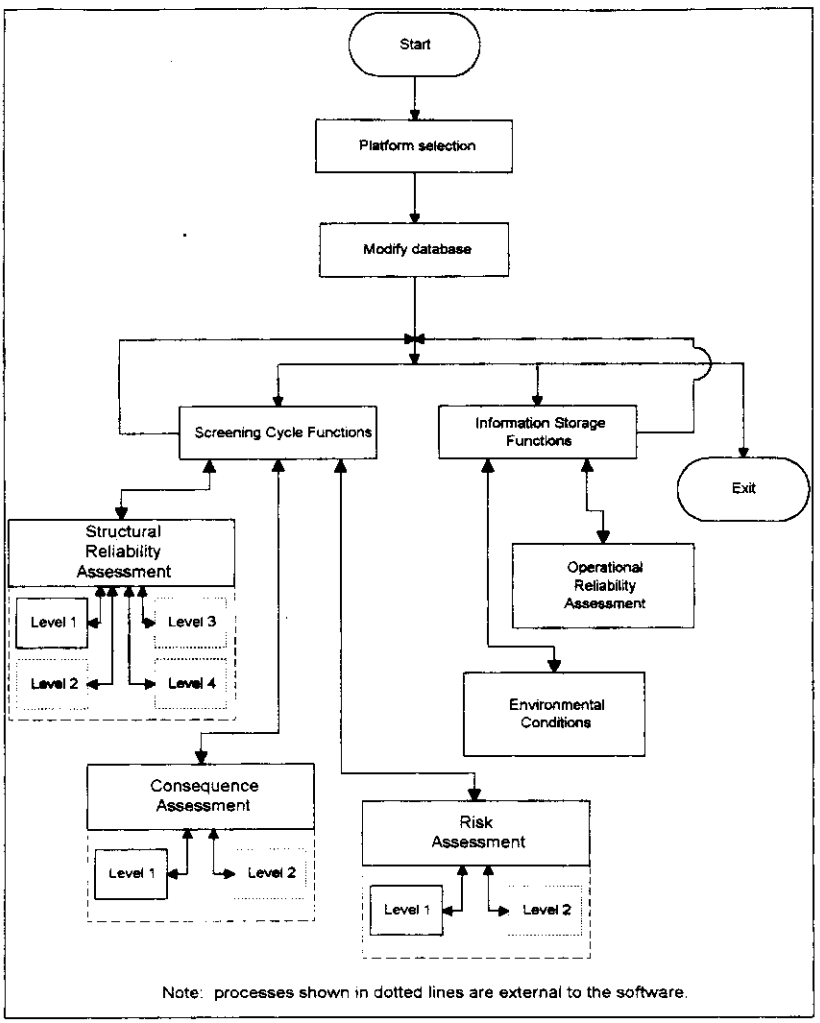

Figure 1. Assessment approach based on appropriate levels of screening detail.

The default level 1 consequence assessment produces a measure of consequences based on a series of questions (Aggarwal, 1991) about manning, crude storage, well safety devices, production levels, and costs. An alternative procedure allows the user to modify these questions; another alternative simply asks if consequence categories (loss of life, spillage, and economics) are considered very low, low, medium, high, or very high. The results of any of these procedures can be considered independently or combined into one consequence measure by one of two methods: utility functions or tabular consolidation.

The level 1 consequence measure is plotted against the level 1 RSR value to produce a visual level

1 risk analysis procedure: The user evaluates the position of the platform data point (based on the values of RSR and consequence measure) versus the given lines of acceptability to determine if the platform is acceptable, marginally acceptable, or not acceptable. The lines of acceptability on this risk assessment chart (Figure 2) are adjustable and should be set according to the equation

$$RSR = \exp\left[\Phi^{-1}\left[1 - P_f\right]\sigma_{lnRS} - \sqrt{2\sigma_{lnS}\Phi^{-1}[2\,(1 - 1/RP) - 1]}\right],$$

where σ_{lnRS} is the estimated standard deviation of the joint distribution of the natural logs of the structure's capacity (R) and the natural logs of the maximum forces to which the structure is subjected (S), σ_{lnRS} is the estimated standard deviation of the distribution of the logs of the forces (S), and capacity (R), RP is the return period of the design loads (usually 100 years), and P_f is the allowable annual probability of failure, as determined by a safety function. (For a discussion of possible safety functions, see Staneff and Ibbs, 1994; Iwan, et al., 1992; and Bea, 1990.)

The adjustability of the assessment process allows the user to tailor the system to suit his or her needs, reflecting the preferences of industry and regulatory representatives for a decision-aiding tool rather than a decision-making tool.

Information Storage

The information storage and management parts of the IMS have two different forms: (1) a data base of platform information, accessible to all present and future assessment procedures, and (2) an advanced graphical interface for the storage and management of environmental data.

The IMS stores the following information in a separate data base file for each platform: name, latitude, longitude, notes, operator, lease number, slots, water depth, miles to land, Lambert East and Lambert North coordinates, installation date, first production date, type, location (region), production (MBBL and MMCF each day), and status. This information is automatically loaded into the IMS calculation routines when a platform is selected for assessment.

Environmental data are entered into the system in an advanced, graphical manner. For example, (Figure 3), after establishing the

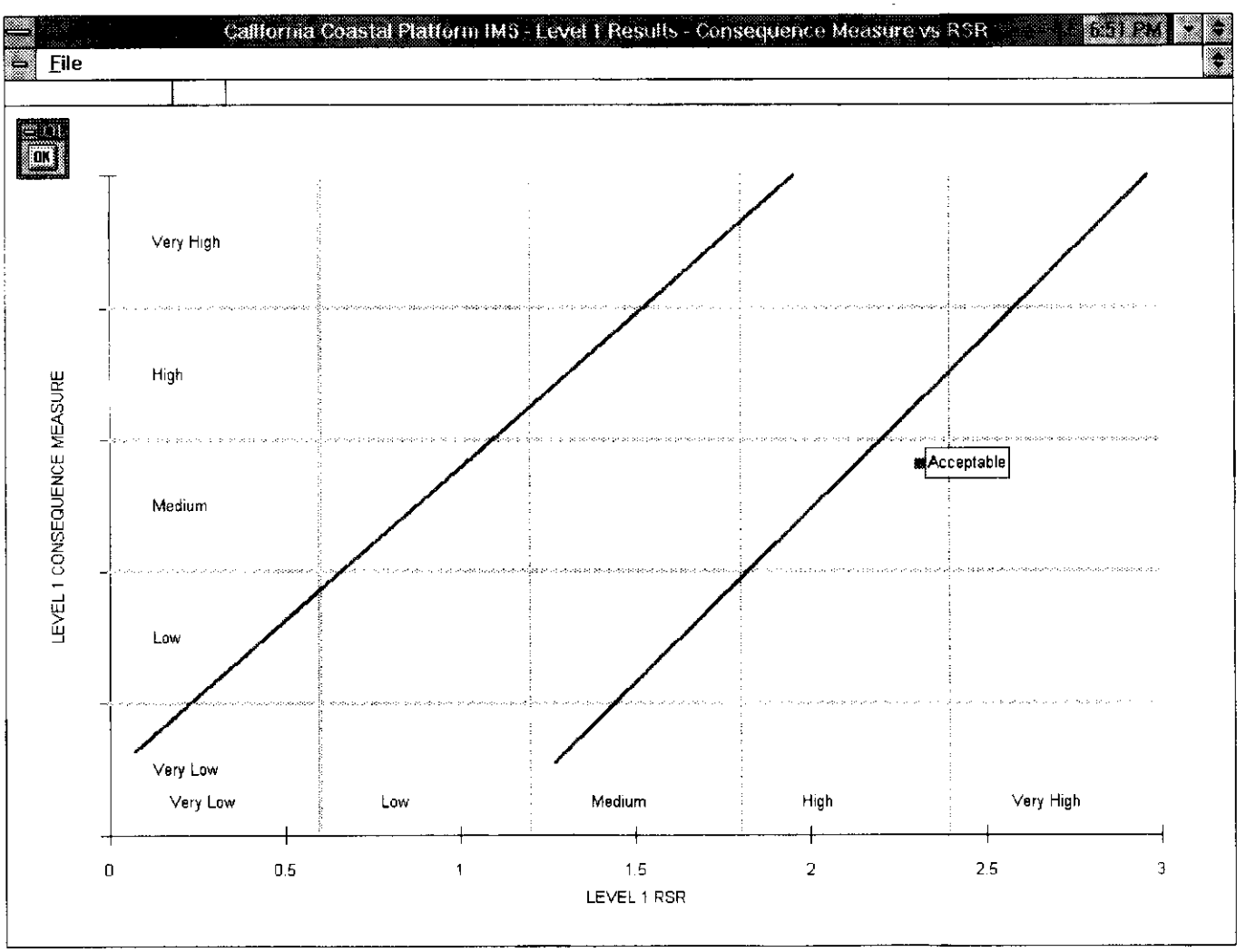

Figure 2. Consequence measure vs. RSR chart.

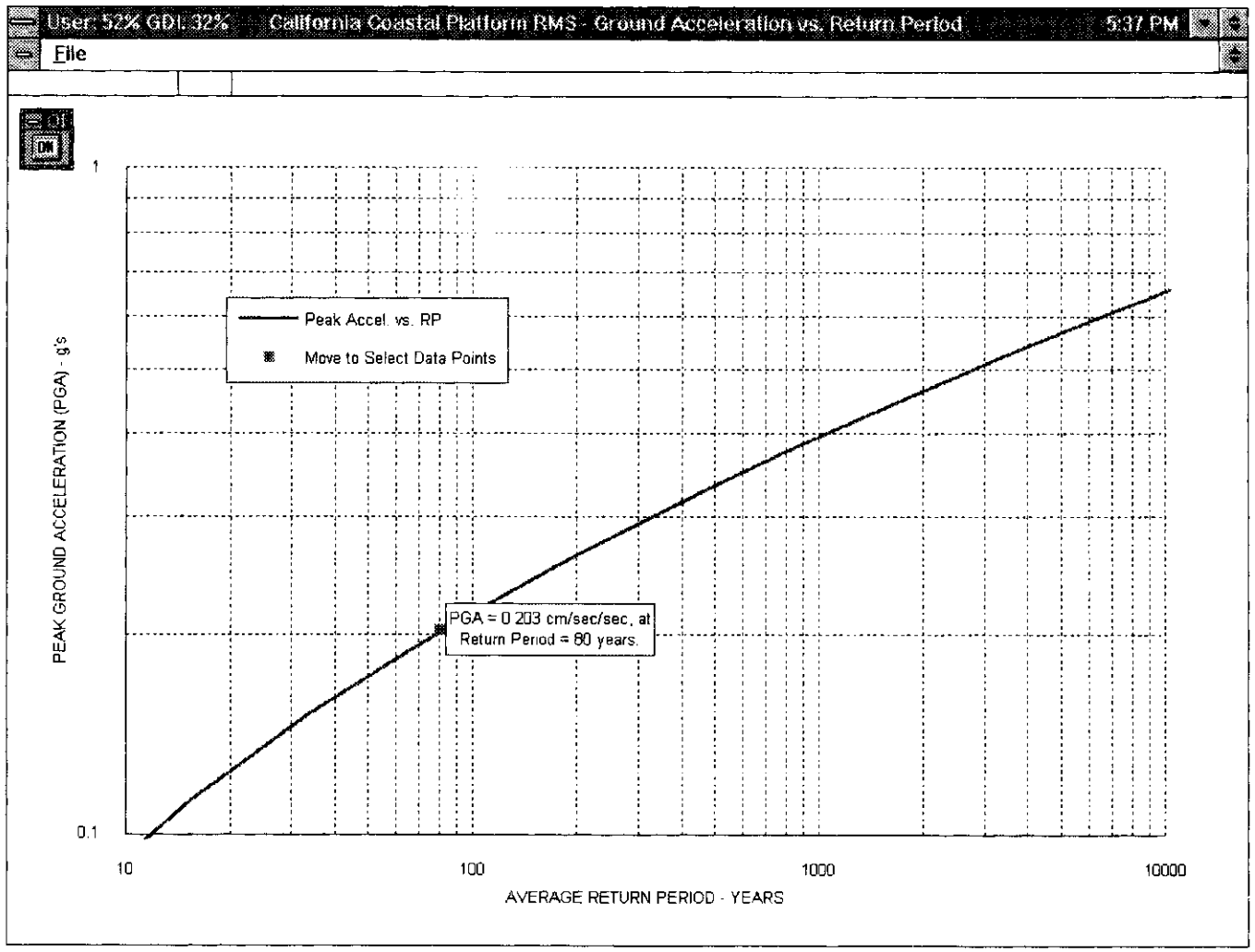

Figure 3. Ground acceleration vs. return period inpout chart.

distribution of peak yearly ground acceleration values, the user moves the platform data point horizontally to select the design return period and vertically, to the curve, to select the design peak acceleration value. A second chart is then presented for the input of the bias of the distribution; a third allows the user to graphically set the ratio of horizontal spectral acceleration to peak ground acceleration, on the basis of the structure's period and its soil type; and a fourth presents the spectral acceleration distribution calculated from the information provided in the previous charts. Moving the platform data point up to the curve, the user sets the design value for horizontal spectral acceleration. A similar procedure is used to determine design values for maximum wave height, wind speed, and current velocity. All input and output data are stored and are ready for presentation or for use in a level 2 structural analysis.

Project Evolution

Throughout the project, technical advice was solicited and received from representatives of both industry and government. The principal investigators, with guidance from the technical advisors, determined that it was not feasible to produce both a knowledge-based expert system and a simplified structural analysis. The structural analysis portions of the project were split off into two other projects (Bea and Craig, 1993; Bea and Mortazavi, 1995). The first has been incorporated into this project (level 1 structural analysis), and provisions have been made for incorporation of the second (a level 2 structural analysis). Further, the technical advisors influenced the philosophical decision to move away from a true expert system (the knowledge-based system) to a decision-support system, giving the user more control over the requalification process and more potential for expansion and modification.

An outgrowth of this research, suitable for future incorporation into the IMS, was research by Staneff and Ibbs (1994) into the mathematical implications of safety standards.

Ibbs and Bea continue to work on the IMS concept. Such work will include simultaneous examination of multiple platforms for comparative purposes, calculation of estimated and actual fleet risk, establishment of safety standards, and comparison

of predicted probabilities of failure with historical values.

Cooperating Organizations

ARCO Exploration and Production
California Coastal Commission
California Sea Grant College System
California Seismic Safety Commission
California State Lands Commission
Chevron Oil Company
Exxon Production & Research
Marathon Oil Company
Mobil Oil Company
Nippon Steel
Noble, Denton & Associates
Norsk Hydro a.s.
PMB Systems Engineering
Shell Oil Company
Texaco Oil Company
UNOCAL Corporation
U.S. Minerals Management Service

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Development of a Model for Steep, Directional Seas

University of California, Berkeley
R/OE-23
1992–94

William C. Webster and Parthasarathy Mamidipudi

The goal of this project was to develop a model to study the dynamics of steep, random deep-water waves. The importance of the problem stems from the need to predict the forces on offshore structures in severe sea-states, which requires a good simulation of sea-state.

Several models exist to predict the evolution of ocean waves. Most are either restricted to simulating "calm" seas, or, if they are able to take into account the full nonlinearity involved in the behavior of steep seas, are very expensive. Furthermore, any procedure that requires a long time simulation encounters the problem of specifying a suitable numerical condition to terminate the computational domain; otherwise, waves reaching this boundary are reflected. By reducing the dimensionality of the problem, Green-Naghdi equations make a good candidate for simulating nonlinear water waves. The equations are highly nonlinear and hence require special integration procedures. In this project, a Thomas algorithm was developed to solve these nonlinear equations. The algorithm was first used to solve for steady wave solutions. Subsequently, it was used to integrate the equations in the time domain.

Steady Waves

The steady-state governing equations were derived from the time domain equations by seeking a solution in a frame moving with a constant velocity. These equations were then differentiated so that the highest-order derivative appearing in each of the equations is the same. A two-dimensional Thomas algorithm was implemented in FORTRAN to solve these equations. The chosen domain was rectangular. The solver is capable of obtaining a steady state solution at any arbitrary angle in the rectangular domain. The wave profile and celerity match those obtained from the two-dimensional equations. An initial guess, usually a linear sinusoidal solution, is specified. A Newton's iteration is performed to progressively improve the solution to nonlinear equations. Since the Green-Naghdi equations allow for rotational solutions, irrotationality was imposed as a criterion to check for convergence in the Newton's iteration loop. The wave celerity is obtained as a part of the solution and is also increasingly improved with each iteration. Figure 1 shows the steady state solution obtained for two different wave amplitudes using the level 1 equations. Figure 2 shows the cross section of the wave profiles for increasing wave amplitudes and the celerity plotted as a function of wave amplitude non-dimensionalized with respect to wave length. Equations in time domain.

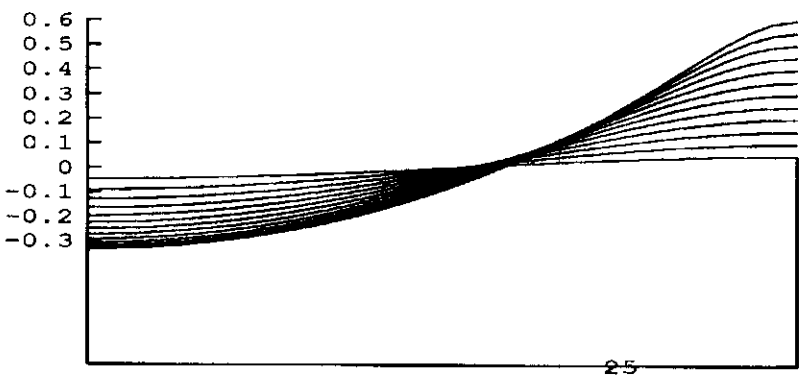

Figure 1. Two different wave amplitudes using level 1 steady state solution.

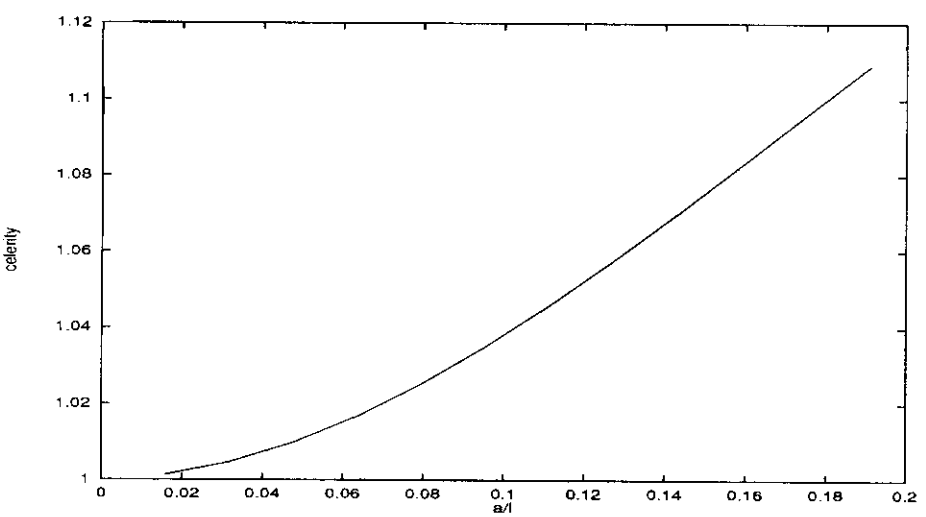

Figure 2. Cross section of steady wave profile and celerity vs. steepness for level 1 steady state solution.

Open Boundary Condition

The implementation of the solution procedure for the time domain is far more straightforward for the steady state because a good initial guess, the solution from previous time, is readily available. The problem we considered was that of a wave maker at one end and either a wall or an open boundary at the other. A two-dimensional algorithm was implemented to solve the three-dimensional problem. First, a simpler case of physical boundaries was considered to truncate the domain: that of an infinitely deep wave tank with walls on all four sides. Next, the more interesting open boundary was considered. In doing long time simulations, it is desirable to deal with as small a numerical domain as possible. Thus, the waves created in the domain should be let out of the domain to continue the simulation. Artificial termination of the numerical boundary is critical because an incorrect boundary condition corrupts the interior solution. Two issues need to be addressed in this regard: nonlinearity and randomness. Most available boundary conditions of the absorption type depend on estimates of the wave celerity at the boundary. This procedure does not work well for random waves. The interaction between different wave numbers also needs to be taken care of in a good boundary condition.

We found that the open boundary condition needs to be addressed before the simulation of three-dimensional steep seas can be achieved. For this purpose, a two-dimensional wave tank with a wave maker at one end and an open boundary at the other was considered. Two types of boundary conditions were investigated and found suitable. The first involved spatial convolution to specify the solution at the terminated boundary, while the second involved time convolution.

In the context of spatial convolution the idea of pseudo-differential operators was introduced to first obtain unidirectional wave type solutions for the linear case. It was found that for small amplitude waves (linear case), the procedure worked very well. Prediction of the phase velocity of each of the components of the random waves generated at the boundary was handled by using a convolution-type solution of the variables. A one-sided linear domain was appended to the nonlinear domain so as to allow long time solutions. The inner solution, the solution in the nonlinear domain, was not corrupted by any spurious reflections from the boundary.

For large amplitude waves, this procedure does result in reflections in the inner domain as a result of the mismatch between the inner nonlinear solution and the outer linear solution. Efforts are currently underway to minimize these reflections. Figure 3 shows the simulated wave train measured at a wave gauge near the end of the nonlinear boundary. The figure compares two domain sizes. The appended linear domain is the latter 200 nodes in each of the simulations. The total domain sizes are 2500 and 700 nodes respectively. The bigger domain result can be looked at as being the numerically exact solution for testing the open boundary condition for the smaller domain. The results show very good agreement.

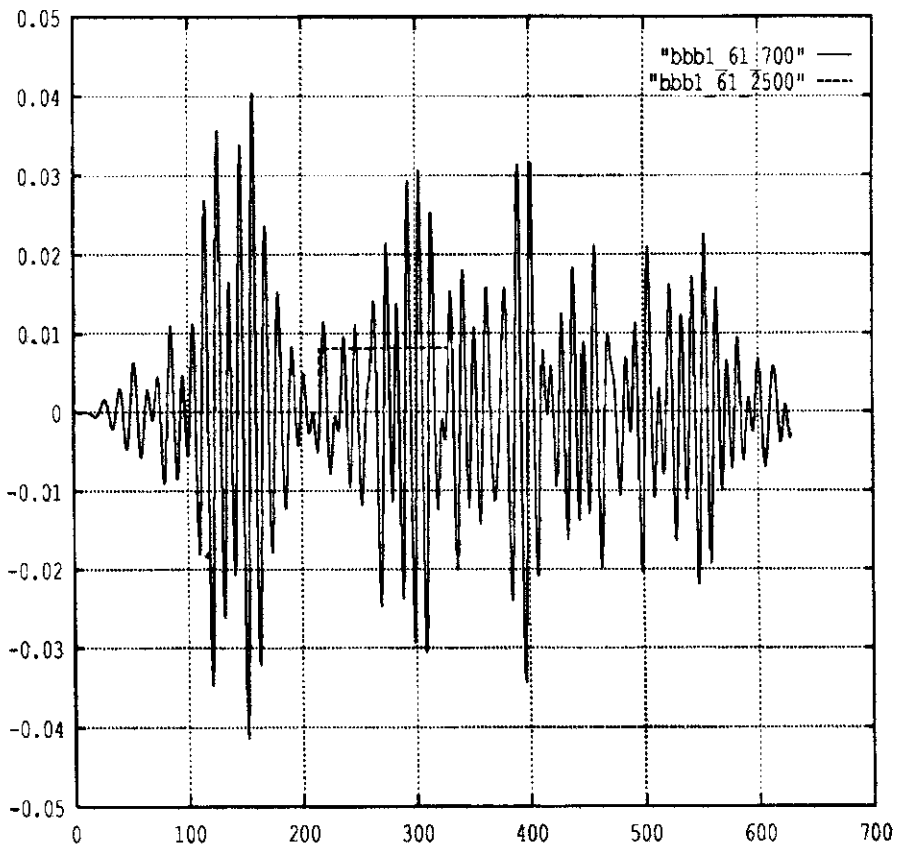

Figure 3. Random wave record at a wave guage, comparing two different domain sizes of 700 nodes and 2500 nodes. The one-sided domain size is 200 nodes. The results are at node 300. The time record is approximately 50 representative wave periods.

The time-convolution solution was also found satisfactory. The boundary condition in this case is the Neumann type. The spatial derivative of the unknown variable is obtained as a time convolution of the solution from starting time to the present. This condition is therefore implicit and is solved in conjunction with the governing equations. The Laplace transform technique is used to obtain the time-convolution solution for the linearized governing equations. The procedure was first tested for Level 1 equations, and the solutions were found to be exact for the small amplitude case. Since the computer time for this evaluation is expected to increase with time, an accelerating procedure was implemented which required the same number of computations at each time step, thus reducing the computer time. Currently, this procedure is being extended to large-amplitude random waves and higher-level equations.

Acoustic Techniques for Remote Sensing of Fish Stocks

Scripps Institution of Oceanography, UCSD
R/OE-24
1991–94

Jules S. Jaffe

Despite the obvious benefits of three-dimensional imaging sonar systems for oceanographic applications, little research has been done on this topic. Only a few documents refer to the advantages of using multidimensional arrays for fish assessment. Extensive research was done on underwater acoustic imaging in the 1970s, but only a little in the 1980s. We think that one of the major results of the 1980s, increased computing power at decreased costs, enables us to implement a host of new applications. Our goal is to apply underwater acoustic imaging to the remote sensing of fish stocks. Here we describe two experiments done with the Fish TV (FTV) imaging system. In one case the sonar was deployed from a remotely operated vessel (ROV) in the vicinity of the Scripps Canyon. In the other case the sonar was used from the research vessel *Flip*. In both cases, successful tracking of multiple animal targets in the field of view of the sonar indicated the capabilities of the system for localizing ecosystems and measuring their evolution.

Canyon Experiment

The experiment near Scripps Canyon was carried out July 9–11, 1993, 1 km west of Scripps. The FTV system was deployed with an ROV from the research vessel *Robert Gordon Sproul*, anchored on the north side of the canyon in 50 m of water. The 150-m FTV cable enabled the ROV to reach the edge of the canyon at a depth of 80 m. Also attached to the ROV were a low-light-level camera; a wide-angle camera for maneuvering; lights with red filters; an instrument to record conductivity, temperature, and depth (CTD); and a fluorometer.

The FTV was calibrated on May 12, 1993, and the calibration was verified during the July cruise using a standard target. The transmitted signal was a 175-msec single-frequency pulse with a bandwidth of approximately 4.4 kHz, centered at 445 kHz. The sonar imaged a volume of water of 5 m^3, starting 0.8 m from the transducer and extending to 4.6 m from the transducer.

Figure 1 shows the minimum detectable target strength as a function of range for a signal-to-noise ratio of 10 dB for the Scripps Canyon experiment. At a range of 4.6 m, FTV detected targets as small as –76-dB target strength. The camera and lights were designed to permit visual identification of animals. The low-light-level CCD camera had a 50-mm television lens attached. It was focused 1.5 m from the ROV. The field of view at this range was 22 cm by 29 cm, with a depth of field of 28 cm. The volume of water in focus was therefore approximately 0.02 m^3 and was in the center of FTV's field of view (FTV's field of view was 42 cm by 42 cm at 1.5-m range). Evidence from optical pigment studies suggests that crustacean zooplankton are insensitive to red light. Therefore, 570-nm red filters were used with the underwater lights to avoid any influence on natural behavior. Video data were stored on videotape and were also tapped off and merged with data from the FTV system monitor. These combined images were stored on videotape. They provide synchronization between the video and the FTV data. The CTD recorder also stored fluorometric readings from the fluorometer.

The imaging system was used in two modes: profiling, and fixed depth. In profiling mode, we measured the density, temperature, fluorescence, and acoustic bandwidth of targets as a function of depth. In fixed-depth mode, we tracked acoustic targets while the

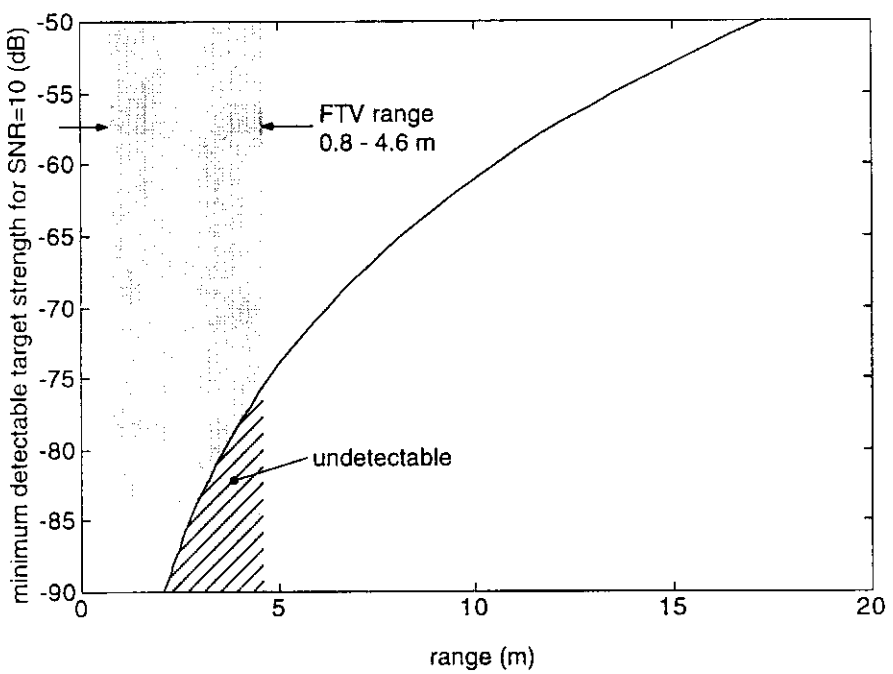

Figure 1. Minimum detectable target strength as a function of range for data taken from 11:06 p.m. to 11:24 p.m., July 10, 1993. A signal-to-noise ratio of 10 dB is assumed. Hatched region indicates undetectable targets according to the criterion.

ROV drifted at a given depth. The intention was to drive the ROV upcurrent to the limit of its tether, then let it drift downcurrent. Data were collected at two rates. When FTV displayed data in real time on the system monitor, the information was saved to disk once every 2.5 sec. When data were not displayed in real time, the information was saved to disk once every 1.4 sec.

Data processing focused on a portion of the data set obtained on July 10, 1993, at a depth of 10 m. Images 404 to 446, (59 sec of data) were examined. Six targets were tracked during this time (Figure 2). The tracks indicate a gross movement of targets away from the transducer at approximately 10 cm/sec. The ROV was at the limit of its tether downcurrent at the time, and it appears from the movement of the targets that the ROV was facing downcurrent, with the targets drifting past. Movement of targets in elevation and azimuth showed a certain amount of correlation, which can be attributed to pitch and yaw of the ROV. We concluded that the ROV worked well as a Lagrangian platform. The integrated environmental package also worked. Using FTV we could simultaneously track multiple targets the acoustical size of euphausiids *in situ* and measure details of their motion.

Open Ocean Experiment on *Flip*

The experiment on *Flip* was carried out March 24–25, 1993, 15 nautical miles southwest of Point Loma, California, in the San Diego Trough. It was a voyage of opportunity for us; *Flip* was going out on a routine training mission and had space available for scientists. *Flip* was towed to the site by the U.S.S. *Navajo* and was put into the vertical position in the early afternoon on March 24. It drifted throughout the afternoon, night, and next morning and was returned to the horizontal position late in the morning on March 25. It was in the vertical position for approximately 22 hours. The experiment had three components: (1) The FTV system was used to measure three-dimensional tracks of zooplankton, (2) a video camera was used to visually identify animals, and (3) a 200-kHz fish finder was used to monitor the position of the deep scattering layer in relation to FTV during vertical migration over the 22 hours of the experiment. The FTV system was mounted on the hull of *Flip* 37 m below the water line, facing away from *Flip* and aimed 20° below horizontal. The FTV system was calibrated February 11, 1993. The transmitted signal was a 170-msec swept-frequency pulse with a bandwidth of approximately 10.3 kHz, centered at 445 kHz. The sonar imaged a volume of water of 4 m^3, starting 1.5 m from the transducer and extending to 5.3 m from the transducer. Figure 1 shows the minimum detectable target strength as a function of range for FTV during the *Flip* cruise. At a range of 5.3 m, the minimum detectable targets are smaller than the target strength of –85 dB, corresponding to crustacean zooplankton less than 9 mm long.

The video camera was on throughout the night, and video data were recorded from 6:02 p.m. on March 24 until 7:52 a.m. the next day, with a gap from 2:00 to 4:00 a.m. The images showed quantities of marine snow drifting past as well as many animals that were so close to the camera they were out of focus. However, many animals were in focus and recognizable because of their shape or their swimming behavior. Among the animals identified were euphausiids, amphipods, copepods, chaetognaths, squid, and small fish.

Ten acoustic sequences were taken at different times during the night, with either 60 or 300 images in each sequence. The total was 2040 images, or 255 megabytes of data, filling the hard disk to capacity. One sequence starting at 9:01 p.m. was singled out for detailed analysis. Although this sequence had only 60 images in it, it was chosen in preference to the longer sequences with a bad receiver channel. Analysis included the usual normalization of beams and matched filtering. Next, the images were processed by using the target identification algorithm. Each target was tracked by using a search algorithm that looked for targets in each image in the vicinity of targets from the previous image. Eventually 314 targets were tracked, some for only 2 images, others for as many as 13 before they disappeared. The acoustic target strengths of the tracked targets ranged from –83.0 dB to –57.7 dB. From the functional regression in Figure 1, this corresponds to crustacean zooplankton 10 mm–66 mm long. The average swimming speed of each of the 314 targets is plotted against its target strength in Figure 3. Mean speed for all targets was 0.11 m/sec. Median speed was 0.10 m/sec.

Over the size range of animals observed, we found no link between animal size, as measured by target strength, and swimming speed. All sizes of animals swam with a broad range of speeds, but the center of the range did not vary with target strength. All sizes of animals also showed the full range of turning behaviors (Figure 3). What can be concluded from these observations? If the target strength axis were extended to include larger nekton, there would be a link between target strength and swimming speed, with larger animals swimming faster than smaller animals. The range of animals observed during the *Flip* observation may simply have been too small to show such a link. As stated earlier, the range of target strengths observed corresponded to crustacean zooplankton 10–66 mm long. Thus, the range of animal lengths was less than an order of magnitude. Also, the larger targets were more likely small fish with strongly reflecting air bladders rather than large crustaceans. This would compress the range of animal sizes even further. We may have been looking at a fairly homogeneous group of animals, in terms of size. Under these conditions, zooplankton of this size class may tend to have the same repertoire of swimming behaviors.

Conclusions

The results of these two experiments show that our three-dimensional sonary system can be used to track animals in the field. This device represents an entirely new

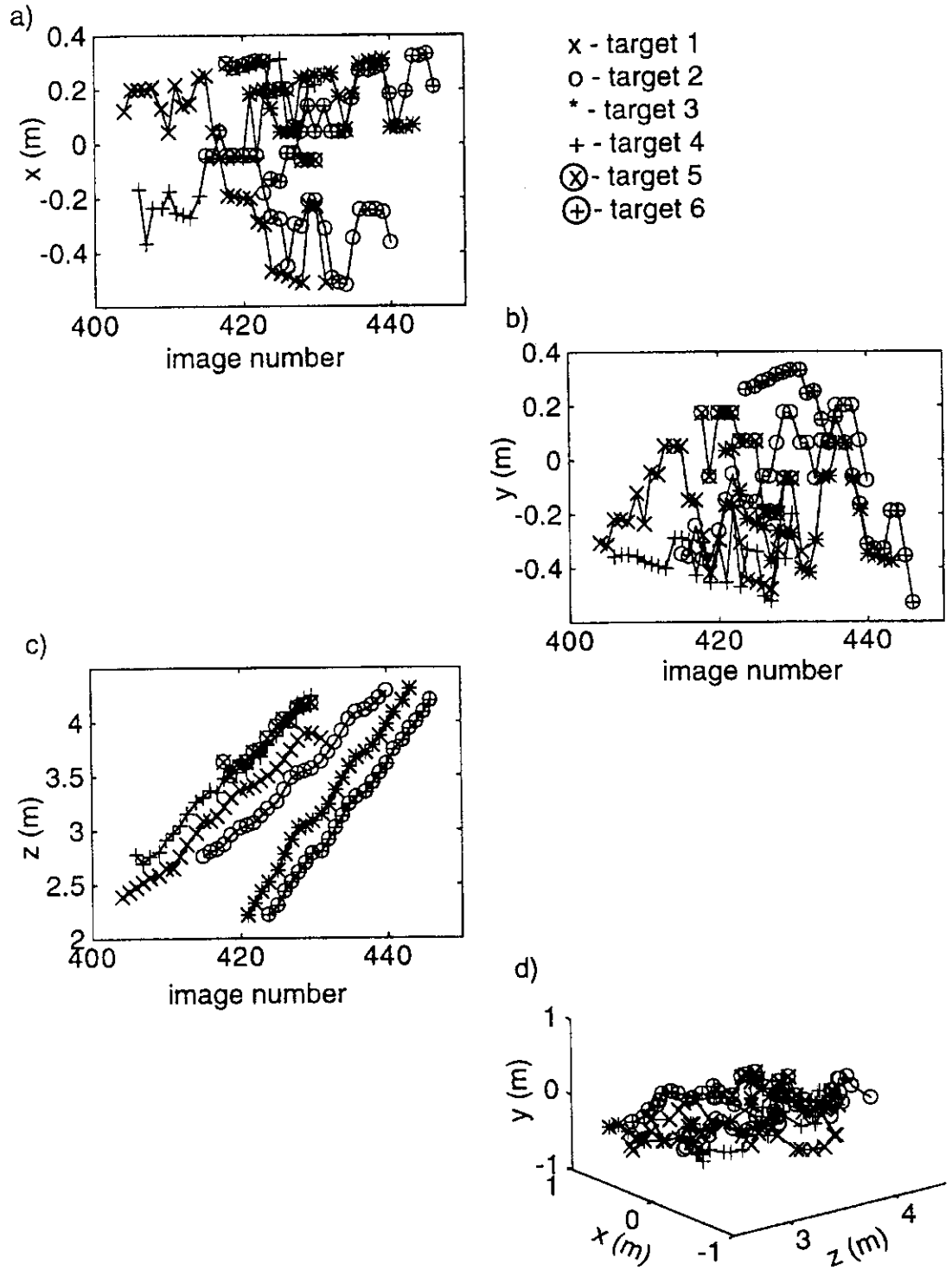

Figure 2. Paths of six targets tracked in a data set from the Scripps Canyon experiment. Time interval between images is 1.4 sec. a, *x*-component of motion. Positive *x* is to the left. b, *y*-component of motion. Positive *y* is up. c, *z*-component of motion. Positive *z* is away from the observer. d, Target paths of the six targets shown in three dimensions.

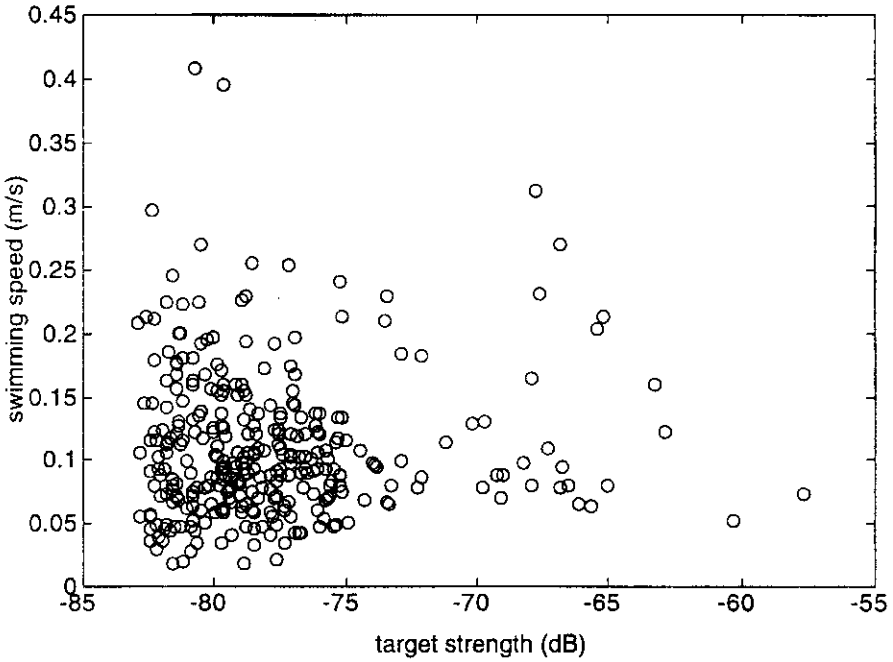

Figure 3. Swimming speed as a function of target strength for 314 targets.

tool for monitoring behavior *in situ*. Error analysis of the data also confirmed that the system can be used to monitor the swimming speeds and densities of pelagic animals in their own habitat. We think, on the basis of our results, that future versions of multibeam, high-resolution imaging sonars can be used to study various aspects of fish schools, including the schools' density and behavior.

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University of California
U.S. Navy—U.S.S. *Navajo*

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Improved Use and Management of the U.S. Exclusive Economic Zone with Advanced Satellite and Geographical Information Systems Technologies

Scripps Institution of Oceanography, UCSD
R/OE-25
1993–94

James J. Simpson, Ali Tajeldin, Robert Keller, and Andrew Schmidt

This report is divided into two major parts. The first part summarizes accomplishments made during the period October 1991 to October 1994. The second describes the functioning prototype that incorporates remote sensing and geographical information systems (RS/GIS system) capabilities. The prototype is currently in beta testing.

Accomplishments

An advisory panel consisting of members of the commercial, sports, and regulatory components of the California fisheries community was created. Christopher Dewees, Marine Fisheries Specialist, serves as chairman. A meeting of the advisory panel at California Sea Grant headquarters in La Jolla in November 1991 highlighted two points: (1) the importance of accurate cloud detection in satellite data and the need for ancillary information from GIS historical data bases to help mitigate periods of no information during clouds and (2) the need to support the management needs of stock assessment and regulatory agencies charged with managing operational fisheries in the U.S. Exclusive Economic Zone. The panel also decided that support of pelagic species would be emphasized in the initial RS/GIS system.

The revised RS/GIS system places stronger emphasis on supporting management of marine fisheries. More emphasis also has been placed on using management-based laboratories (e.g., Southwest Fisheries Science Center, National Oceanic and Atmospheric Administration [NOAA]) in the beta-test evaluations of the system.

Phase I implementation of the system is complete. Specific data bases included in the phase I implementation are NOAA's high-resolution data base of global conductivity, temperature, and depth (CTD) and the world bathymetry and coastline data base. Major map projections also are supported. The phase I RS/GIS system was beta-tested by several groups (e.g., University of Washington Applied Physics Laboratory). Numerous additions, adaptations, and changes to the phase I system were implemented as part of the beta testing. Finally, several international organizations (e.g., the United Nations Food and Agricultural Organization, the ICES program) have expressed interest in possible use of the RS/GIS system.

The Revised RS/GIS System

The CTD Oceanographic Rendering and Analysis Laboratory (CORAL) is a general-purpose X-Window/OSF motif-based platform that encapsulates data sets and filters for manipulating the data. CORAL provides the user with several noteworthy features: (1) a consistent, powerful, graphical user interface to data bases and related sets of filters; (2) a simplified method for manipulating subsets of data from one or more data bases; (3) a high-level data base interface for acquiring any desired subset of data; and (4) a user-extensible menu system to give maximum flexibility for both the analysis and rendering of selected data.

The CORAL system takes advantage of the device independence of the X-Window system (Jones, 1989; Johnson and Reichard, 1990) to ensure use with a wide variety of hardware and software configurations. Furthermore, the OSF/Motif widget set (Young, 1990; Open Software Foundation, 1991) used in CORAL's graphical user interface provides an intuitive set of graphical mechanisms that give CORAL the same "look and feel" as many other existing Motif applications. All CORAL application filters were implemented in C programming language, UNIX shell scripts, or both. These choices were made to ensure compliance with the open architecture and device independence of X-Window.

CORAL was designed to overcome common difficulties associated with the use of large data sets and to enhance the usability of such data. The difficulties include, but are not limited to, (1) the lack of a standard format for data; (2) the need for a compact representation for large-volume global data sets; (3) the ability to add, correct, search, and retrieve subsets of data from global data sets efficiently and effectively; (4) the ability to efficiently analyze data; (5) the need to make the analysis libraries highly user-extensible with minimum user effort; (6) the need to render analyzed results by using a large number of different map projections (e.g., Lambert equal area); (7) the need to incorporate ancillary data (e.g., bathymetry, topographical relief, vegetation maps) into the data processing where appropriate; (8) the need to develop a flexible and intuitive graphical user interface that could be used to eliminate much of the tedium associated with applications involving large data sets; and (9) the need for device independence and network communication of data and analyzed results. Such difficulties must be overcome if meaningful symbiotic analyses between remotely sensed (e.g. GEOSAT altimeter) and earth-based observations (e.g., oceanic CTD information; fish-catch statistics) are to be performed on a global scale. The need for such joint analysis of large-scale data sets has been amply demonstrated (Earth Observing System, 1989).

CORAL System Overview

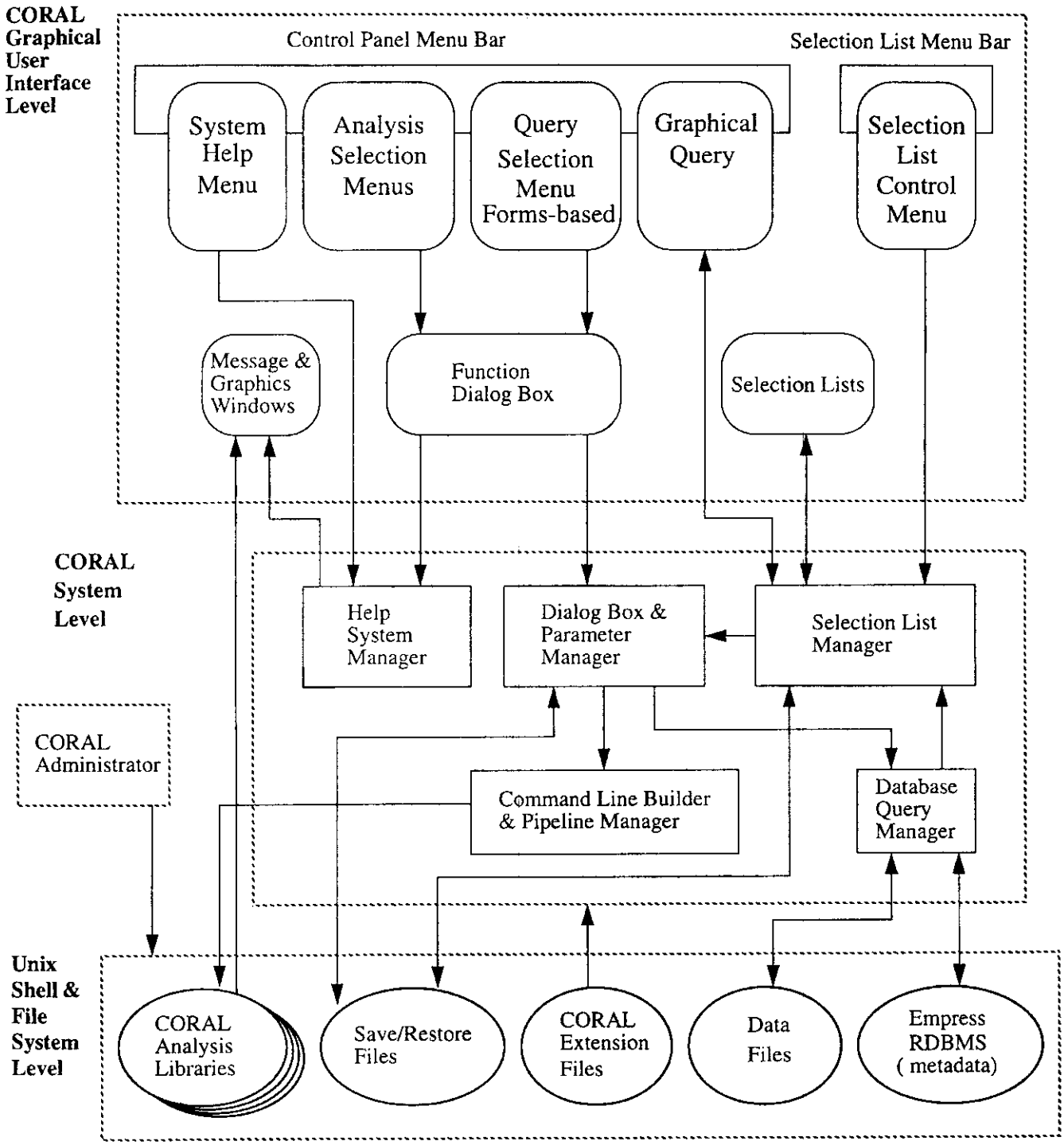

Figure 1. The hierarchical structure of CORAL consists of a graphical user interface, the CORAL system level, and the UNIX shell and file system level.

CORAL is a hierarchical system (Figure 1). Level 3 is the CORAL graphical user interface. The interface provides cascading menus for help in using the system, selecting applications, and choosing queries. An important structure of the interface is the CORAL multiple selection list and selection list control consoles (Figures 2 and 3). These data structures are used to control and manipulate diverse data flows (e.g., environmental oceanographic data, fisheries data) within the CORAL system.

Results from queries are returned to the selection list. Once there, they can be manipulated by the user. The graphical user interface also provides a control menu for the selection list, sets up application dialog boxes, and provides user-friendly access to message and graphic windows. Level 2 of the hierarchy corresponds to the CORAL system level. Here, all CORAL management functions are performed (e.g., data base query, help system manager, command line builder, and pipeline manager). Level 1 of CORAL contains the relational data base files, CORAL extension files, save/restore files, and the CORAL applications libraries. It corresponds to the UNIX shell and file system level. Level 0 corresponds to the UNIX kernel level. Users have access to CORAL through the CORAL graphical user interface and command line builder. Only the CORAL administrator has direct access to all CORAL level 1 and level 2 data and control structures.

The CORAL system allows user-specified command-line applications to be interfaced with and run from the CORAL graphical user interface. The interface between the CORAL graphical user interface and the command-line application is specified with CORAL's three types of ASCII files: the CORAL configuration file, which allows the user to individually configure the graphical working environment; the application list file, which describes the structure, contents, and help text of CORAL's pull-down menus; and the application definition file, which describes the parameters, help text, and interface of the CORAL application dialog box. CORAL runs the user-defined applications by creating a command line based on information in the application dialog box and executing the command line via a UNIX system statement.

The method of adding applications to CORAL described here has several advantages: (1) Applications can be added to CORAL without recompiling the CORAL system. (2) Applications can be run in the background; in fact, CORAL can run several applications at the same time. (3) Applications that crash do not cause the CORAL system to crash. (4) CORAL can take advantage of existing UNIX filters. (5) Because each CORAL application can be executed by using the command line, applications can be executed outside the CORAL environment. This last feature can be useful during the development of an application, or if complex shell scripts or job scheduling are needed.

CORAL provides, for example, support for CTD and hydrographic applications in ocean science. Thus, the global NODC high-resolution CTD data base (Figure 4) and the WDBII global bathymetric data base are the primary data bases in CORAL. Support for a variety of data sets of information on conditions off the west coast of the United States (e.g., high- and low-resolution CTD data) also is provided. A summary of CTD ingest, editing, manipulation, and analysis filters currently in the CORAL CTD applications library is given in Simpson et al., 1994.

For illustrative purposes, CTD data from the ALCAN cruise were selected from CORAL's data base to perform a few of CORAL's analysis functions. From these data, vertical sections of spiciness through an eddy (Figure 5a) and a vertical section of geostrophic flow through an eddy (Figure 5b) were constructed by using the CORAL applications library and command-line builder.

These simple examples illustrate how a scientist might use CORAL to do a desired scientific analysis. First, data would be selected from the data base by using appropriate criteria entered through the cruise-query and cast-query applications. Next, data would be piped through a set of analysis filters (e.g., pressure, temperature, and salinity → density → specific volume → geostrophic velocity or spiciness), and finally results would be rendered by using an appropriate map projection. Flexible data structures provide support for the creation of sophisticated scientific analysis filters that can be made completely compatible with CORAL's streaming model. Alternatively, the user could examine the frequency distribution of the data retrieved from a given search before analyzing the data.

Because CORAL is a generalized encapsulator, it can be configured to support a wide variety of applications in the earth sciences. For example, using satellite data to classify clouds according to cloud type requires several different types of inputs. Some of these data are obtained directly from the satellite (e.g., cloud albedos, cloud temperatures), other data are derived statistically from the image data (e.g., textural measures), and still other data consist of expert knowledge (e.g., training sets). CORAL can provide a convenient framework to manage and analyze these diverse data and make them readily available to some supervised form of classification (e.g., decision trees, neural networks).

Estimation of snow water equivalents, useful in hydrological studies and flood forecasting, requires the use of both satellite data and aircraft-based measurements of gamma ray radiation over broad snow-mapping windows (Carroll and Carroll, 1989). CORAL's extensibility provides an efficient and effective basis for encapsulating this type of application, with its diverse data and application filter requirements, into a straightforward, user-friendly analysis protocol.

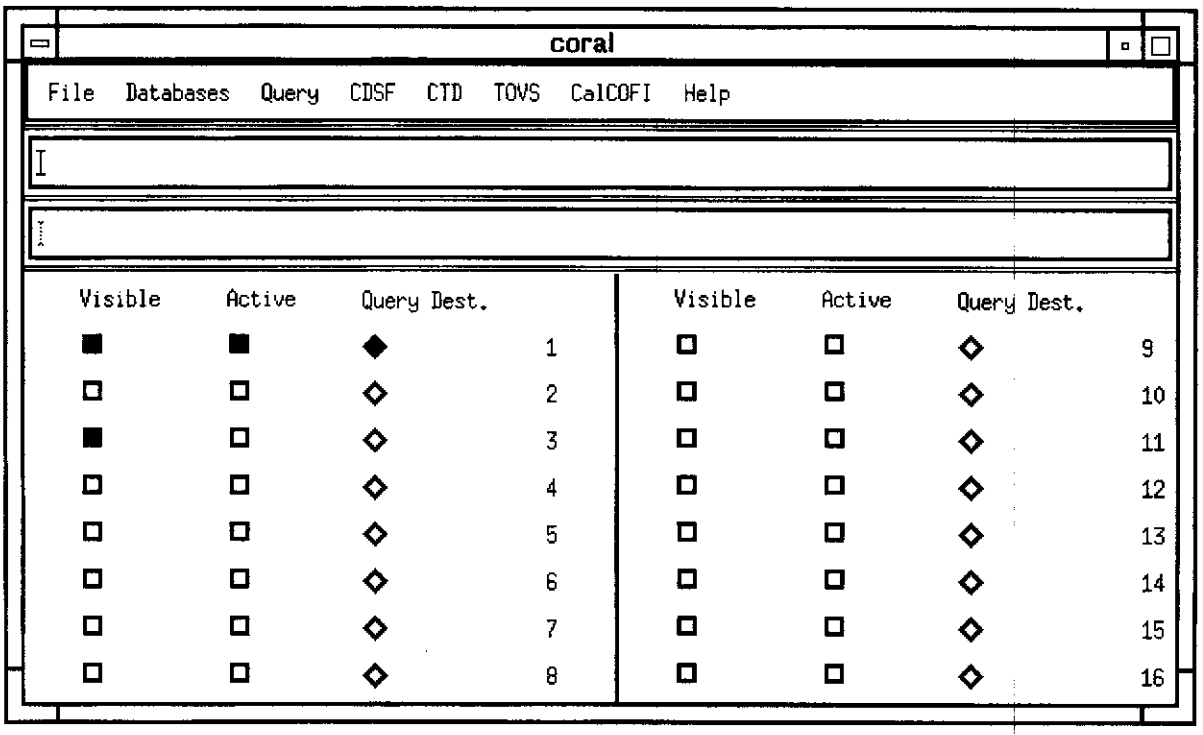

Figure 2. CORAL's selection list control panel shows active and inactive selection lists.

Discussion and Conclusions

Although the version of CORAL described here encapsulates CTD and hydrographic data and related filters, the underlying CORAL design is sufficiently flexible to include a wide variety of other types of data and libraries of associated data-specific application filters (e.g., fisheries and environmental data, recruitment and mortality models; clouds and radiation balances).

The internal structure of CORAL provides a graphical user interface consisting of a cascading menu system, a help system, application dialog boxes, a selection list control console, and multiple selection lists. Extensibility is provided through CORAL's three ASCII file structures: the CORAL configuration file, the application list file, and the application definition file. The same structures also allow a user to custom configure the menu structure of the graphical user interface to his or her personal tastes and needs. Graphically based queries provide an intuitive way to retrieve data from CORAL's relational data bases. Results from queries and application descriptions can be saved and restored. CORAL provides a pipe and command-line builder that constructs and runs executable UNIX programs. Message display and error trapping are provided.

The design philosophy behind CORAL is quite simple: to provide the applications user with a flexible, extensible encapsulating platform for accessing an arbitrary type of data with its associated analysis and rendering filters within the context of a UNIX and X-Window system. We think that currently this is the only way to ensure execution compatibility on a wide variety of hardware and software configurations.

Because CORAL can encapsulate applications for both regularly (e.g., satellite) and irregularly gridded (e.g., CTD) data, it has potential application for both calibration of satellite data with ground truth data and joint analysis of data collected by remote sensing and *in situ* methods. Both these application areas are important for large-scale studies of global changes (Earth Observing System, 1989). Moreover, the relational data base capabilities of CORAL, especially when used with systems for locating and retrieving selected satellite scenes from archived data sets (e.g., Simpson and Harkins, 1993) provide a straightforward way of assembling the specific time-space sequences of data needed for study of a particular global change. Likewise, because CORAL has fully incorporated the X-Window protocol into its overall design, it also provides a device-independent way for several scientists working on a given project to easily share their data and analyzed results.

The basic model of CORAL is to select a subset of data and then run the data through one or more filters. This can be thought of as a stream model. The user normally begins by selecting data from the selection lists, and piping the information through one or more application filters. The stream ends in a graphics file, a data file, a terminal output, or the selection list. For example, the user might select a transect of stations from a CTD cruise and pipe these data through the spiciness filter and then into the vertical section filter, which terminates the stream by displaying a graphics plot of a vertical section of spiciness on the screen and saving the plot in a graphics file (Figure

Figure 3. A typical CORAL selection list containing metadata that describes the results of a query.

5a). Another example is using the cast query filter to find casts with a depth of more than 5000 m in a given geographical area during a particular time. The user selects the cruises of interest via the selection list. These are then given as input to cast query, which finds casts in these cruises that are more than 5000 m deep. The stream terminates when the resulting casts are placed in the selection list or rendered and plotted (Figure 4b). In summary, CORAL, from a user's point of view, provides the ability to easily and quickly locate specific data from large data bases, and subsequently analyze, render, and plot georeferenced results efficiently and effectively. In general, such capabilities should significantly enhance the ability of the earth sciences community to address issues associated with complex global changes.

Cooperating Organizations

National Oceanographic and Atmospheric Administration, Southwest Fisheries Science Center, La Jolla

National Weather Service, Alaskan region, Anchorage

University of Washington, Applied Physics Laboratory, Seattle

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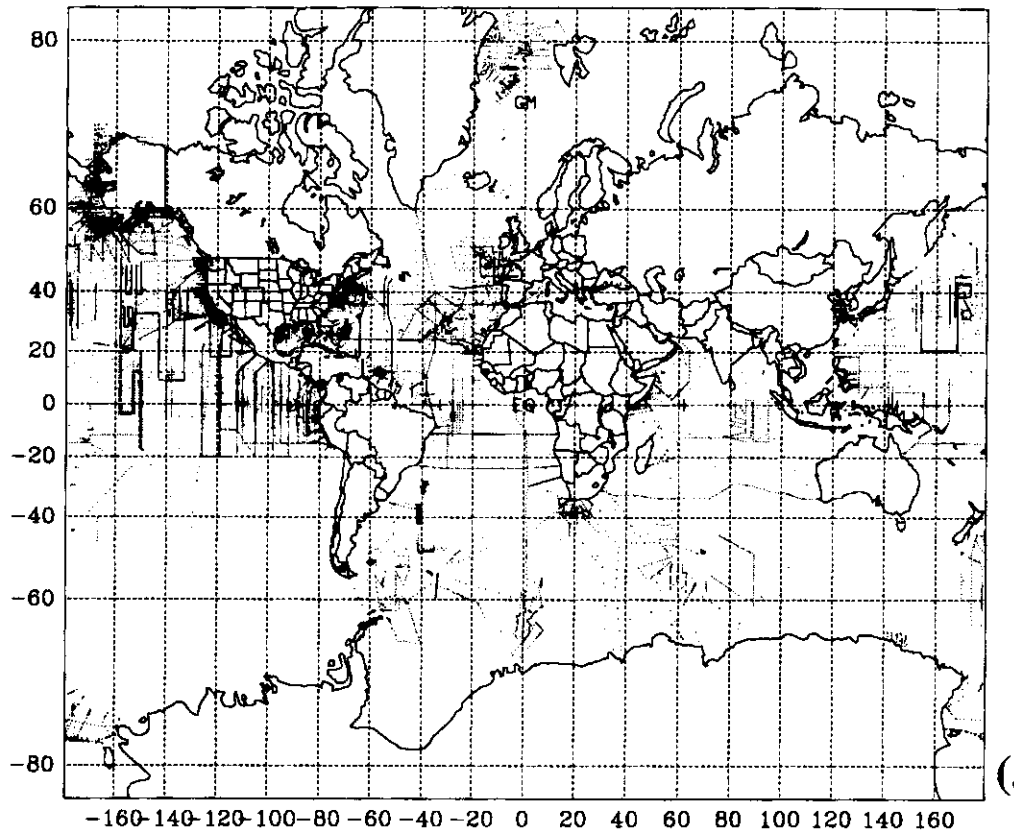

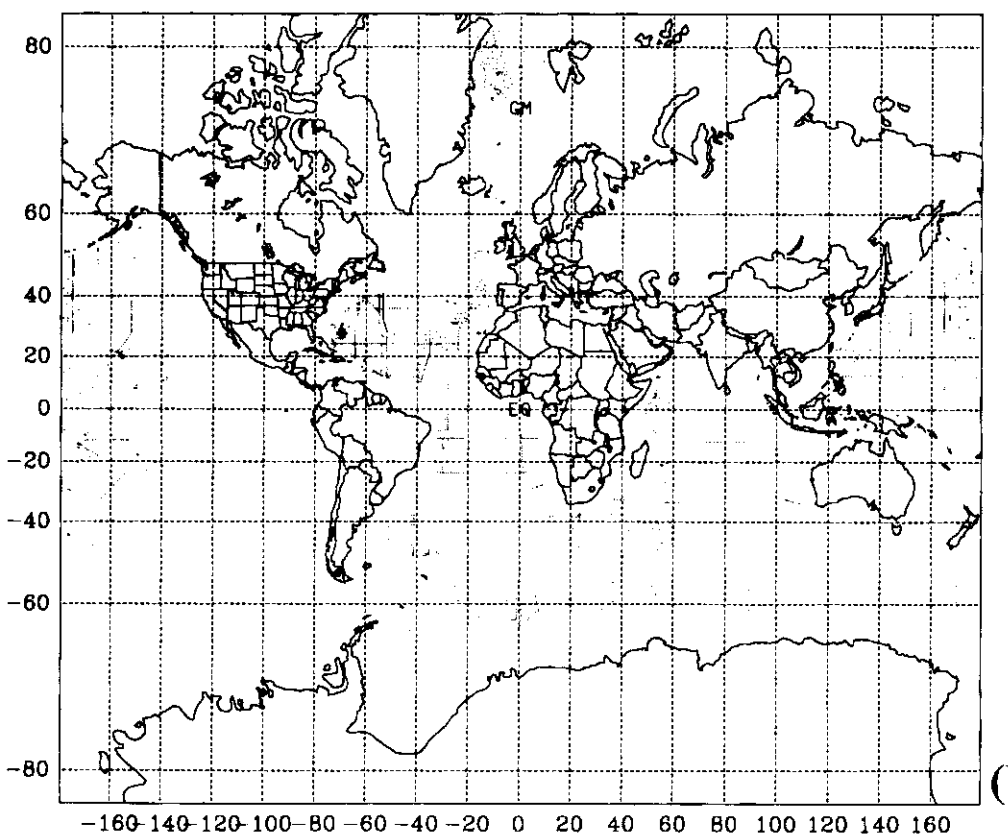

Figure 4. (a) The NODC high-resolution CTD data base currently archived in CORAL. A dot corresponds to a given CTD station. (b) Data in (a) that satisfy the depth criterion specified by the user (see text).

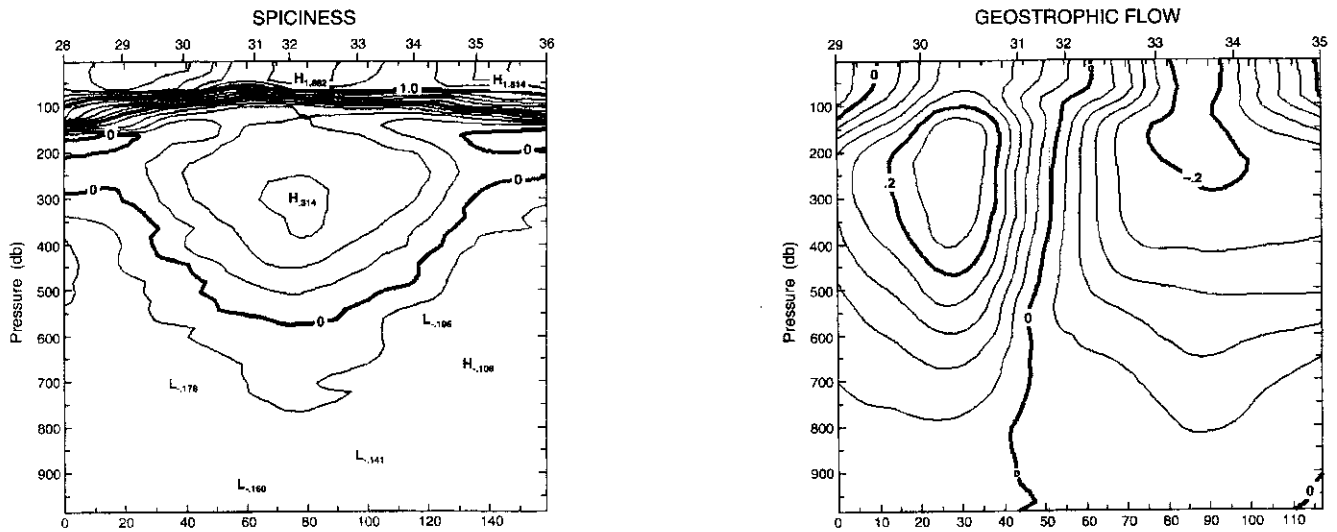

Figure 5. Example of CORAL-rendered analysis for (a) vertical section of spiciness through a mesoscale eddy and (b) a vertical section of geostrophic flow through a mesoscale eddy. The CORAL pipe summaries used to produce these results were (a) spiciness | vertical section and (b) geostrophic velocity | vertical section. Specifics for each filter in the pipe summary are automatically retrieved from that filter's application dialog box.

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Offshore Single-Point Mooring Systems for Import of Hazardous Liquids

University of California, Berkeley
R/OE-26
1992–95

William C. Webster, Wei Ma

This study focused on determining the feasibility, reliability, and safety of offshore single-point mooring systems. The study had three parts: the feasibility and reliability of using a single-point mooring system offshore California (completed by Bea and Salancy, 1994), the development of analytical procedures for determining the forces and motions of the mooring system (completed by Ma and Webster, 1994; Webster, 1995), and an investigation of the safety of chain systems (completed by Ma, 1995). A detailed discussion of the feasibility study was presented in last year's report and is not repeated here. The following discussion centers on the new work completed this year: tools for the analysis of the design of moors, and a detailed analysis of falling chain.

Single-point mooring systems are used to secure a storage tanker in position, and the integrity of the system is important. Failure of the mooring system can cause not only a loss of the station-keeping ability but also other severe consequences, such as oil spillage. One possible mode of failure is the accidental dropping of a chain. The drop may occur because a link in the chain breaks or during other operations in the open ocean, such as connection of two pieces of chain or anchor handling. In the latter case, falling chain could collide with nearby existing installations or expensive underwater facilities.

Because of the fundamental difference between the dynamic behavior of the mooring system before and after failure, this study was split into two parts. Each part was based on a distinct analytical model constructed for the specific purpose. The first part focused on the dynamics of an intact mooring system; the second on the dynamics of the chain after failure.

Part One: Approach Based on the Continuous Model

The formulation of the dynamics of the intact mooring system was based on a continuum model that treats the chain as a continuous, elastically extensible structure with bending rigidity. This formulation was an extension of the theory introduced by Garrett (1982) for the dynamics of slender rods. The formulation can be used to predict nonlinear motions of marine mooring lines. Various loadings encountered in submerged marine structures are included in this formulation. The loadings include the forces on the rod due to hydrodynamic effects resulting from the relative motion between the rod and the exterior fluid (estimated here by using the Morison equation), the weight of the rod, and the effect of pressure gradients in both external and internal fluids (the rod is perhaps hollow and filled with fluid). In addition, the model incorporates bending rigidity, a feature that extends the scope of its application. The motion of the rod is treated by a finite-element approach and incorporated into an efficient computer code. Each finite element has 16 degrees of freedom for three-dimensional computations and 12 degrees of freedom for two-dimensional computations. Thus, each element is much more complex than, for example, a typical beam element in many standard finite-element analysis packages. The high efficiency of this approach stems from the formulation of the elements in inertial coordinates rather than in element coordinates, as is usual in typical finite-element codes. As a result, no rotation matrices are used in the procedure, even when the rod forms a loop or pretzel shape.

The theoretical formulation was computerized into a computer program, CABLE, which was written in standard FORTRAN 77 language. The program carries out a time-domain calculation of the motion and tension of cables and can be operated on a personal computer as well as a workstation. A user's manual of the program was included as chapter IV of the report by Ma and Webster (1994).

The computer program was used to perform simulations of a catenary anchor leg mooring (CALM) system. Two primary ocean environmental conditions were studied. One was the condition of 2-year return period wind, wave, and current. Under this condition, the dynamic analysis of the CALM system with a San Diego class tanker was performed. The other environmental condition was the condition of 100-year return period wind, wave, and current. In this case, the dynamic tension of the CALM system only was analyzed. Salancy (1994) provides a detailed explanation of the environmental conditions used here.

Analysis of the dynamic tension of the CALM system was based on a procedure that combines the guidelines in API RP 2FP1 (American Petroleum Institute, 1987) with our research on the cable dynamics (Figure 1). After several design iterations, statistical data of the tensions of the most loaded line were obtained (Table 1).

Part Two: Approach Based on the Discrete Model

The continuum model developed in the first part is valid when the tension in the chain is above a threshold level ("taut chain"), a condition that is always true when the mooring system is in its integrity. A certain amount of pretension is applied to ensure that the tension in the chain is above that threshold level. This assumption, however, is

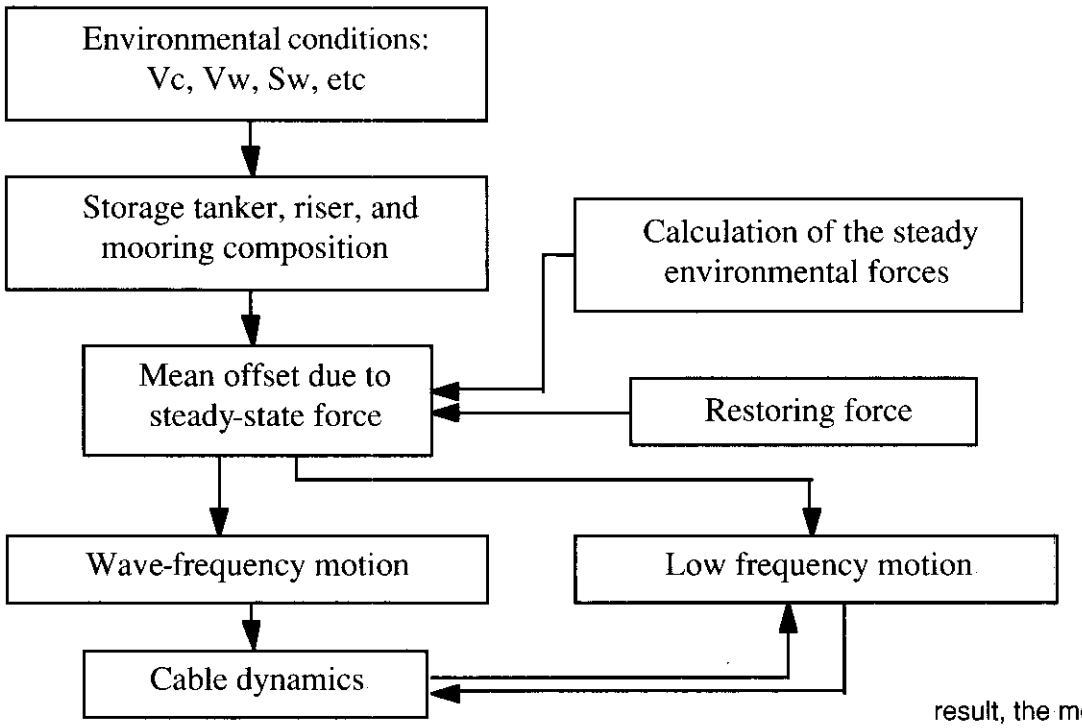

Figure 1. General analytical procedure for analysis of mooring.

Table 1. Tension of the CALM system

Tension (kips)	CALM System + Tanker	CALM System Alone
Mean	98	73
Mean + low frequency	100	80
Maximum	102	96

Note: CALM = catenary anchor leg mooring.

invalid when a chain fails and falls under its own weight. There is a fundamental difference between the behavior of the chain before and after its failure. The dynamic behavior of the chain in the free fall exhibits high tension and low tension in different parts of the chain. The chain in low tension should not be regarded as a continuum, but rather as a discrete system of links with many degrees of freedom. Each link behaves as a rigid body with constraints imposed on its rotational and translation motion (or sliding). A discrete model can account for motions of individual links with respect to the adjoining links, such as sliding, in addition to the motion of the entire chain system. A continuum model, by not incorporating the possible sliding and rotation of individual links, does not include all the degrees of freedom existing in the chain system.

The formulation adopted in the study of the chain in free fall was based on Kane's method (1961, 1983), which includes the relative rotational and translation motion among the links. This is a relatively new method that recent research of rigid-body motion has shown is a good model for falling chain, especially when the tension in the chain is at a low level. This formulation was further expanded to account for the hydrodynamic effects and the effect of the seafloor. On the basis of the formulation, an efficient computer program was developed. The program makes use of the repetitive geometry of the chain system. As a result, the motion of a link can be expressed in a recursive way in terms of the motion of all of the links below it.

In this part of the study, we investigated the sensitivity of the behavior of the falling chain to several important factors, such as the tangential drag coefficient C_T, the number of links N, and sliding.

The nonlinear analysis of the dynamic behavior of the falling chain without sliding between links was investigated first. Because of the random trajectories induced by instability, many simulations of identical configuration were necessary to develop statistical characteristics of the motion. Ten simulations were done for each configuration. The following results were obtained.

- C_T, the tangential drag coefficient, is important. C_T is an empirical value for the evaluation of the hydrodynamic force along the length of the chain. Data are insufficient to assert with confidence the exact value of C_T for a complicated geometrical shape such as chain links. Hence, a range of approximated values was chosen on the basis of the C_T value of a cylinder of equal volume and equal length. The following results were obtained:
- Both the mean radius, measured as the excursion from the vertical, and the falling speed are

the largest for the top link.

• When released from rest, the motion of the chain becomes unstable upon reaching a critical falling speed. This speed, attained when the weight of the link approximately equals the drag force, is sensitive to the value of the tangential drag coefficient, C_T. The smaller the value of the drag coefficient, the larger is the critical velocity.

• Because of instability, the chain does not fall straight. Rather it executes a random trajectory about the vertical. The mean radius of the motion of the top link is sensitive to the tangential drag coefficient. A small drag coefficient, by allowing a greater falling speed, does not allow the chain to develop large excursions from the vertical. Hence, small C_T values result in small mean radii.

• A long chain always has a larger mean radius of motion than a short chain does. Having more links than the shorter chain, the longer chain is more likely to develop an unstable oscillatory motion.

The effect of the sliding was incorporated into the numerical formulation. We found that sliding has a significant effect on the behavior of a free-falling chain. The motion of the falling chain without sliding is less smooth than that with sliding. Sliding can be considered a factor that modifies the damping coefficient of the system. In this case, the sliding force acts as a nonlinear damping force. It allows yet another possible motion for the link to the extent of the "dead-band" with the use of springs with high spring constants. This results in a smoother motion of each individual link in response to the force acting on the link. Each link is allowed to slide along the dead-band in addition to the rotation motion. The dead-band prevents the links of the chain from piling up one on top of the other.

Cooperating Organizations:

ABS Americas
Arco Marine Inc.
Arctec Offshore Corp.
BP Oil Co.
California Department of Fish and Game
California Sea Grant College System
California State Lands Commission
Chevron Petroleum Technology Co.
Chevron Shipping Co.
Exxon Shipping Co.
IMODCO, Inc.
LOOP, Inc.
Marine Safety Office
Naval Civil Engineering Laboratory
Noble, Denton and Associates, Inc.

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Marine Affairs

Managing the Cumulative Impacts of Development: An Implementation Study of the California Coastal Act

University of Colorado, Boulder
R/MA-35
1993–95

Charles Lester

The broad objective of this project was to evaluate the effectiveness of the California Coastal Act in managing the cumulative impacts of development and to provide policy recommendations about managing these impacts. The evaluation was based on a case study of coastal zone management in the Monterey Bay region of Central California, including land use regulation by the California Coastal Commission; Santa Cruz and Monterey counties; and the cities of Santa Cruz, Capitola, Marina, Seaside, Sand City, Monterey, Pacific Grove, and Carmel.

The specific components of the evaluation included statistical assessment of a 100% sample of coastal development permits from 1973 to 1992; legal and policy analysis of development and regulatory case studies; assessment and case study analysis of the implementation of local coastal programs; personal interviews with coastal policymakers in the Monterey Bay Region; and development of recommendations for future enhancements to management of cumulative impacts in the coastal zone. The working hypothesis of the study was that incremental, case-by-case decision making and amendment of plans had undermined the comprehensive resource-protection goals of the California Coastal Act, thereby contributing to adverse cumulative impacts in the coastal zone.

Results

In general, the hypothesis was not supported by the results. The analysis showed that although case-by-case decision making may undermine comprehensive resource protection policies in certain circumstances, incrementalism is also qualified by multiple, crosscutting factors. Moreover, in many respects, the California coastal zone management program achieved its greatest successes in managing cumulative impacts in the Monterey Bay region through incremental yet aggressive regulatory decision making.

More specifically, the analysis showed that successful management of cumulative impacts in the Monterey Bay region was a function of multiple factors, including the following: strong, statewide cumulative impact policies; aggressive, consistent, and cohesive staff implementation; the jurisdictional scope and perspective of the decision maker with the regional perspective being most rigorous; changes in state and local politics; levels of public participation; availability of comprehensive planning and determination of resource impacts; adoption of strong local land use plans; the effective use of conditions and project modifications to mitigate impacts; postdecision monitoring, data collection, and enforcement mechanisms; incremental beneficial and adverse changes to local plans; whether a regulatory body had sufficient geographical and substantive policy jurisdiction to manage impacts (e.g., a watershed); and effective application of the results of assessment of cumulative impacts. Broadly speaking, management of cumulative impacts was most successful when a regional body had both planning and regulatory authority over coastal development.

Discussion

Under the California Coastal Act, management of the cumulative effects of coastal development on resources relies on local governmental regulation directed by local coastal plans. These plans are to be developed by local governments in consultation with the California Coastal Commission and may only be certified by the Commission if they are consistent with the statewide resource protection policies of the Coastal Act. Before completion of local coastal plans, coastal regulation is the responsibility of the Commission. Once the plans are certified, coastal permitting authority is returned to local government, subject to appeal to the Commission, monitoring after certification, and periodic review of local decision making by the Commission. Local governments may also amend their local coastal plans, subject to approval by the Commission.

The analysis of this project showed that coastal permitting in the Monterey Bay region has gone through four major phases. From 1973 to 1976, coastal development was regulated by the California Central Coast Regional Commission (CCCRC) under Proposition 20, a public initiative passed by the voters in 1972. From 1977 to mid-1981, the CCCRC regulated development and began the process of preparing local coastal plans under the California Coastal Act of 1976, a state law that continued the original efforts begun under Proposition 20. This act also required the phasing out of the CCCRC in anticipation of the local coastal planning phase of the Coastal Act being complete. Although no local coastal plans were actually completed in the Monterey Bay region by 1981, all permitting authority was transferred to the statewide California Coastal Commission, and the regional commission was abolished in mid-1981. The length of this interim third phase of state-level permitting varied for particular jurisdictions, depending on when a local coastal plan was finally certified (city of Marina, 1982; Santa Cruz County, 1983; Sand City, 1984; city of Santa Cruz, 1985;

Monterey County, 1988; and city of Capitola, 1990). By 1990, more than 90% of coastal development decisions were being made by local governments.

Management of the cumulative effects of development in the coastal zone was one of the primary purposes of the original coastal laws in California. Case analysis and statistical assessment showed that between 1973 and 1981, the CCCRC increasingly applied strong cumulative impact policies in priority resource contexts. Evidence is provided by both differential processing (cumulative impact cases receive more attention) and relatively high denial rates of development proposals in Big Sur, the Elkhorn Slough watershed, and other nonurbanized settings. In addition, the CCCRC was particularly aggressive in regulating proposed subdivisions in an effort to minimize the increasing residential densities in non-urban areas of the Monterey Bay region. The CCCRC also made special efforts to limit the cumulative impacts of development on such sensitive resources as the Marina Dunes complex, the Asilomar Dunes habitat in Pacific Grove, and the habitat of the endangered Santa Cruz long-toed salamander. Finally, the CCCRC strictly regulated urban development that might contribute to cumulative effects on public access to the beach and recreational resources, such as the redevelopment of the Cannery Row and Wharf areas of the city of Monterey. In contrast, less aggressive regulation was applied to the development of single-family homes and other residential areas in built-out or urbanized settings.

The CCCRC was also active in the early assistance and facilitation of planning exercises designed to incorporate the comprehensive policies of the Coastal Act into local plans. Primarily because of this aggressive regional action, strong plans were adopted for many of the jurisdictions in the Monterey Bay region. In some cases, such as northern Monterey County, significant reductions in overall levels of planned development were achieved. In addition, the CCCRC spent significant time developing plan policies that would, in theory, lead to decreased cumulative impacts on resources. Much of this work was rooted in studies sponsored by California Sea Grant (e.g., Dickert, 1976) and centered on the idea of determining the carrying capacity of various resource "sheds" (e.g., watersheds, groundwater basins, viewsheds, traffic areas, recreational and beach areas).

With the transition to statewide permitting under the California Coastal Commission in 1981, the overall intensity of the regulatory process in the Monterey Bay region has decreased significantly: fewer permits are processed in regular public hearings, public participation by regional actors has decreased, and permit denials have dropped markedly. These changes occurred simultaneously with the election of a more conservative, antiregulation governor, who placed extreme political and budgetary pressures on the Commission, and with the gradual approval of the Santa Cruz County local coastal plan and significant parts of the Monterey County local coastal plan, both of which more clearly specified what types of development could be permitted, thereby decreasing the number of controversial projects being heard. Although strong policies for management of cumulative impacts are incorporated into local coastal plans, the overall effect of these factors is a decrease in the regulatory intensity of the coastal program of the Monterey Bay region relative to the CCCRC phase.

Approval of local coastal plans began a transition to the fourth and current phase of coastal zone management in the Monterey Bay region. Analysis of coastal permitting under the plans suggests that the context for coastal regulation is continuing to evolve. First, most regulatory decisions are now being made by local zoning administrators or planning commissions. In general, these forums are subject to less public oversight with respect to goals for protecting regional resources. Rather, regulatory denials and conditions are shaped more by traditional local issues of land use planning such as neighborhood conflicts and growth patterns. Even still, denial rates for coastal development increase relative to the statewide permitting period. Although significant coastal development projects were still subject to appeal to the California Coastal Commission, appeal activity dropped slightly through the 1980s. Finally, available data also support a finding that locally regulated coastal development is more likely to be granted a variance—a common local zoning practice—to particular resource protection policies than was the case under a regional or statewide coastal permitting authority.

Finally, another dimension of the implementation of local coastal plans that may be undermining the comprehensive goals of the Coastal Act is the incremental amendment of the plans. Aggregate analysis suggests that a significant part of the amendments are proposed to facilitate particular development projects. However, the California Coastal Commission has conducted little comprehensive oversight or monitoring after certification of local coastal plans, and thus little information is available to judge whether the implementation of local coastal plans is providing effective management of cumulative impacts on resources. Interviews with local coastal zone managers suggested that local governments are not giving cumulative impact concerns the same attention as was given to the development of local coastal plans and regulation at the regional level. Part of the reason for this appears to be quick overturn in staff, limited opportunities for coordination between the California Coastal Commission and local planning staffs, and the natural focus of local planners on issues of local scope.

Conclusion and Recommendations

Attention to the management of cumulative impacts may be increasingly attenuated in the transition to local regulatory implementation. Local governments have a natural focus on local issues. Plans are subject to development-driven

change, and variances in particular cases are easy to come by. In addition, the movement away from the broad resource protection goals of the Coastal Act is made worse by lack of rigorous oversight and evaluation by the California Coastal Commission of local regulatory practices.

The lesson of the successful management of cumulative impacts by the CCCRC is that strong resource management depends on a clear connection between comprehensive planning processes and regulatory decision making. This connection requires feedback loops from the incremental decisions and associated resource impacts of the regulatory process to the broad context of recognized resource planning goals and resource contexts.

Currently, little planning is taking place at the regional or statewide level that might reinvigorate the coastal management process in the Monterey Bay region. Moreover, regulators are not giving sufficient attention to the monitoring of individual decision-level data for the purposes of more comprehensive management. In the Monterey Bay region, the California Coastal Commission still has many of the original staff members that participated in the days of regional coastal management. This situation contributes greatly to the maintenance of broad regional resource protection goals in the regulatory decisions and local coastal plan oversight decisions that are still made by the Commission. It is important to reinvest in the planning mission of the California Coastal Commission though, so as to take advantage of the experience of this staff and to instill in new coastal zone managers the legacy of management of cumulative impacts in the coastal program of the Monterey Bay region.

The Commission should begin targeted evaluations of local coastal plans that build on the baseline data and analysis of the initial planning phases. Efforts should be made to redefine resource sheds, carrying capacities, priority cumulative impact concerns, and indicators for more rigorous monitoring after the certification of local plans in each plan's jurisdiction. Finally, because of limited resources, attention should be focused on maintaining existing urban and rural boundaries and nonurban densities.

Cooperating Organizations

California Coastal Commission, Central Coast District Office

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Rapid Response

Interaction of Wave-Driven Nearshore Flows with Cross-Shore Barriers and Tidal Jets

Scripps Institution of Oceanography, UCSD
R/CZ-121PD
1993–94

John L. Largier

The exchange of water and associated matter to and from the nearshore is poorly understood. Transporting, among other things, pollutants, larvae, nutrients, sediment, and swimmers, cross-shore flow is important for water quality, aquatic ecology, transport of sediments and recreational activities in the nearshore region. Observation of energetic three-dimensional circulation, such as rip currents, suggests that this exchange may be rapid, but the transport across the breaker line is of little consequence if the material from the nearshore merely recirculates back into the surfzone. Anecdotal observations of color lines and plankton distributions suggest that a line of minimum cross-shore exchange may be found only a few surfzone widths from the shore. If this is the general case, pollutants may be trapped in the nearshore area and larvae and nutrients trapped offshore. However, compared with our knowledge of the propagation and dissipation of waves in the nearshore region, we have only the crudest knowledge of the wave-driven cross-shore circulation. An understanding of these flows both on uninterrupted planar beaches and in the strong flows found near barriers to longshore flow such as jetties and groins is important.

The overall objective of this project is to investigate the strong, persistent cross-shore flows found in the vicinity of barriers (jetties) to wave-driven alongshore flow. This aspect of the cross-shore flow was chosen because barrier-enhanced flows likely account for the largest part of the cross-shore exchange and because of the environmental, economic, and engineering importance of tidal inlets. Flows near barriers are also easier to observe than those on a planar beach, making them an obvious starting point in the long-term project of understanding cross-shore flows in all shoreline configurations. A second aim of this project, in association with a companion project on the flushing of small boat harbors (California Department of Boating and Waterways), is to investigate the interaction of these wave-driven flows with the tidal flows to and from smaller bays and harbors.

It is expected that offshore flows at barriers occur in all conditions, except when the incident waves are exactly normal to the coast, a rare occurrence, especially in the Southern California Bight. By investigating flow patterns and dynamics under a variety of wave and tide conditions, this project addresses the effect that tidal flows may have on flushing the nearshore region and, conversely, the effect that wave-driven nearshore flows have on the flushing of bays and small harbors. In both cases, the underlying motivation is to improve the water quality of the coastal waters off California.

Our approach is to investigate the circulation around barriers through a description and analysis of the Lagrangian flow field, that is, through the deployment and high-resolution tracking of reliable drifters, drogued at a variety of depths. The entrance to Mission Bay in San Diego was selected as a geometrically simple, economically important, and logistically appealing site. Although we chose a simple example, this work aims to address the circulation patterns in a general sense; barriers to longshore flow may be a groin on a planar beach or an irregular headland on a rocky coast. Observations will also be obtained from areas with geometric configurations different from those of the typical armored entrance represented by Mission Bay. Experiments will involve the deployment of drifters upstream of the jetty (to describe the longshore flow), at the jetty (to describe the offshore deflection of the flow), and at the end of the jetty (to describe the flow beyond the controlling wall). Basic questions to be answered are, What are the current speeds? How broad and deep is the flow? What is the amount of offshore transport, and how far offshore does it extend? Does the offshore flow recirculate into the surfzone, continue alongshore beyond the barrier, or continue in an offshore direction?

This 1993–1994 rapid response project had two major objectives. The first was to complete development and testing of a unique scientific instrumentation that is to be the primary source of data in subsequent studies: a fleet of differential global positioning system (GPS) Lagrangian drifters. The second was to make preliminary observations of the flow at the north jetty of Mission Bay.

The Lagrangian drifter system has been designed, built, and tested. Final adjustments to make the drifters rugged enough for possible transit through the surf zone are being made.

We have made two preliminary field deployments at the mouth of Mission Bay. The findings indicate the strong, but complex, flows associated with a wave-driven rip current on the outer edge of the channel jetty and a tidal jet between the channel jetties. In the first study, we used drifters without instrumentation to qualitatively survey the flow in the study area. Drifters were deployed on an ebbing tide along Mission Beach, near the jetties, and in the channel between the jetties. Drifters deployed off Mission Beach, north of the channel, were transported into the channel by way of a seaward rip current and a landward tidal flow into the bay. For the

second deployment, we used fully instrumented differential GPS drifters to observe the flow both inside and outside the channel. On the day of deployment, however, strong winds dominated wave-driven flows. The drifters described a tidal jet being deflected by a southerly offshore current, presumably set up by the wind. Water originating nearshore off Mission Beach moved offshore and southward along the outer edge of the deflected tidal jet. The Mission Bay effluent moved southward and then onshore at Ocean Beach in a large counter-clockwise eddy. Selected drifter tracks are shown in Figure 1. This deployment indicated some physical weaknesses in the drifter system, which we are now rectifying. This preliminary description of velocity and length scales is being used to plan the intensive field work for 1995.

Our drifter fleet is the first to use the latest differential GPS technology for high-resolution observations of nearshore fluid dynamics. Our preliminary observations indicate that much small-scale structure is not well described by lower resolution drifters that were developed for large-scale offshore studies. This drifter technology is currently being used by other groups in the development of the next generation of GPS Lagrangian drifters for oceanographic use, allowing descriptions of mixing processes offshore. The "offshoot" development is funded by the Office of Naval Research and it is likely to see deployment in the study of the Santa Barbara Channel, funded by the Minerals Management Service. In addition, plans are being discussed for deployment of these drifters in collaborative studies in San Francisco Bay (dispersion of pollutants), Sacramento–San Joaquin Delta (erosion of levees), Grays Harbor (recruitment of Dungeness crab), Tomales Bay (biogeochemical balances), and in Monterey Bay and the vicinity of Point Reyes (larval dispersal and plankton blooms).

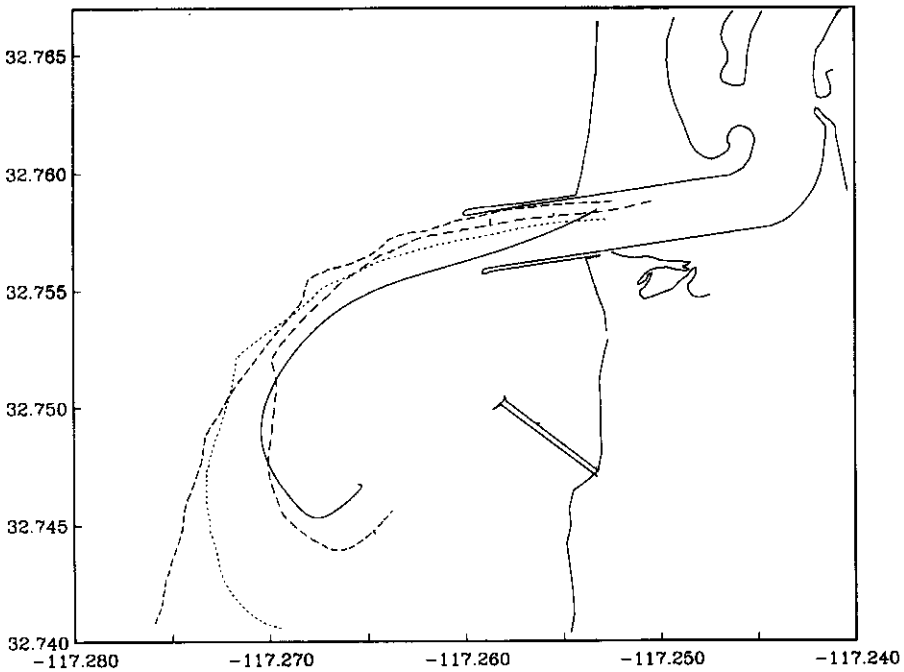

Figure 1. Mission Bay outflow drifter tracks observed on October 7, 1994.

Cooperating Organizations

California Department of Boating and Waterways
City of San Diego Lifeguard Service
NRaD
San Diego County Communications Department
U.S. Navy Postgraduate School

Coprostanol as a Chemical Probe to Assess Sewage and Toxic Pollutant Inputs in Southern California Basins

University of California, Los Angeles
R/CZ-126PD
1994–95

M. Indira Venkatesan

Introduction

The contamination of ocean water by sewage is a major concern of agencies responsible for water quality, resource management and public health and recreation. It is, therefore, of paramount importance that we be able to trace and monitor sewage plume in aquatic systems. Toxic pollutants being discharged from sewage outfalls off major urban centers are also of concern.

This project attempted to construct historic profiles of inputs of sewage carbon and toxic contaminants (i.e., DDTs) in the Santa Monica and San Pedro (SM/SP) basins in order to determine correlations with recent improvements in wastewater treatment and also to provide baseline data for future improvements. Further, the project attempted to test the hypothesis that the relative amounts of coprostanol and DDTs in sediment core sections should reflect whether the origin of DDTs was from sewage, resuspension, or toxic-waste dump sites in isolated marine locations. The project thus directly addressed Sea Grant priorities by determining the distribution and fate of sediment contaminants and then assessing multiple impacts in the coastal areas of Southern California.

The compound classes measured were sterols (including fecal sterols —coprostanol and epicoprostanol), DDTs and other chlorinated pesticides, and PCBs; these compound classes, with the exception of sterols, were targeted in NOAA's National Status and Trends (NS&T) Program. In addition, trialkylamines (TAMs), important constituents of fabric softeners which serve as good tracers of sewage carbon, were measured by collaborators from Spain.

Methodology

Sediment cores were carefully collected in 1991 using slow-entry Soutar box cores in eight locations (four each from SM and SP basins from a water depth of ~300 to ~900 meters, Figure 1). They have been age-dated by Pb-210 measurements (C. Huh and M. Baskaran, personal communication, 1993) and date back to about 1850. The sediment horizons (0.5 cm cuts) correspond to 2 to 4 years in recent times. Pb-210 measurements reflect undisturbed surface sediment recovery, which was corroborated by the presence of flocculent layers on the top. In some of the cores, sections were available for analysis which post-dated the shut-down of the Hyperion 7-mile sludge pipeline in 1987. Further, analysis of such fine-resolution core sections provided an unique opportunity to correlate the historic trends of sewage sterols with those of toxic contaminants.

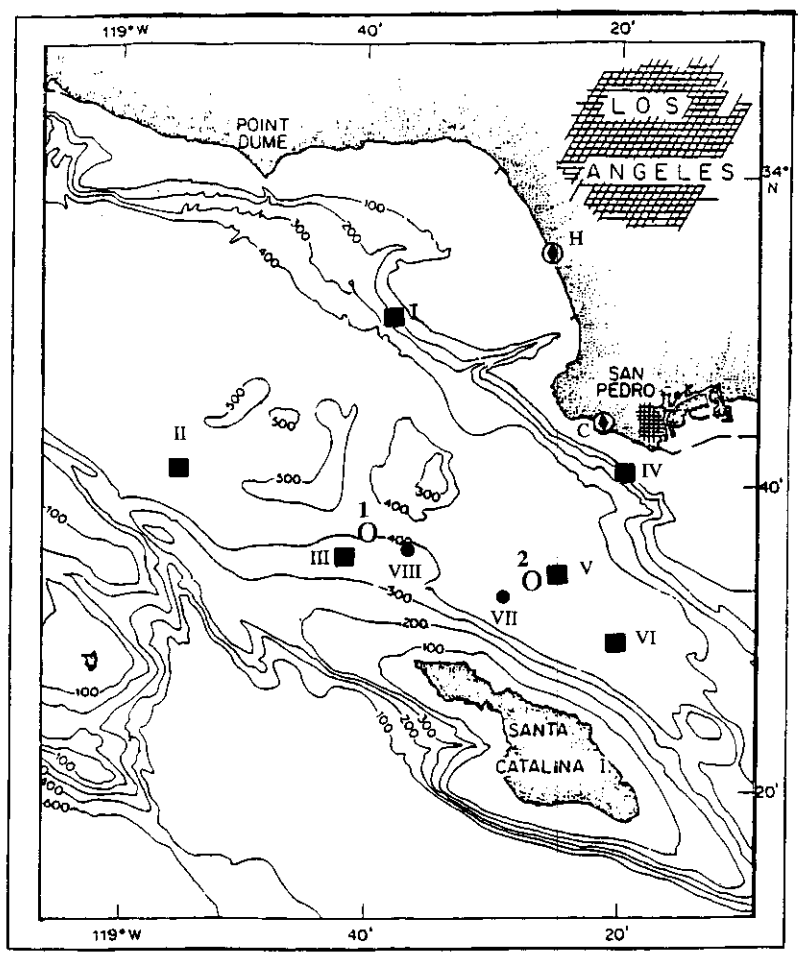

Figure 1. Box core locations.
■ I–VI: NOAA, NSAT cores
● VII–VIII: new cores
O 1,2: dumpsites
◍ H = Hyperion, City of Los Angeles, and C = Los Angeles County Sanitation Districts

Sterol fractions were analyzed in about 100 sections from eight cores. Chlorinated pesticides and PCBs were measured from two cores. Six cores had already been analyzed for chlorinated hydrocarbons (pesticides and PCBs) as part of the NS&T Program. A few effluents and some sediments collected at discharge points from Los Angeles city (Hyperion) and county (JWPCP) outfalls were also analyzed. Sterol fractions were purified (Venkatesan et al., 1987) and derivatized into silylethers with BSTFA (with 1% TMCS) and quantified by gas chromatography (GC) on a fused silica capillary column (DB-5); identification was confirmed by GC/mass spectrometry (Venkatesan et al., 1986). 5α-androstan-17β-ol was used as an internal standard. Effluent samples were extracted with methylene chloride in a separatory funnel and processed as above. Methodology and QA/QC protocols are the same as those employed in the NS&T Program (Venkatesan, 1994). The alkylamines (TAMs) were analyzed in cores I–VI by collaborators (Bayona and Maldonado) from Spain using a GC/nitrogen–phosphorus detector, following the method of Chalaux et al. (1992).

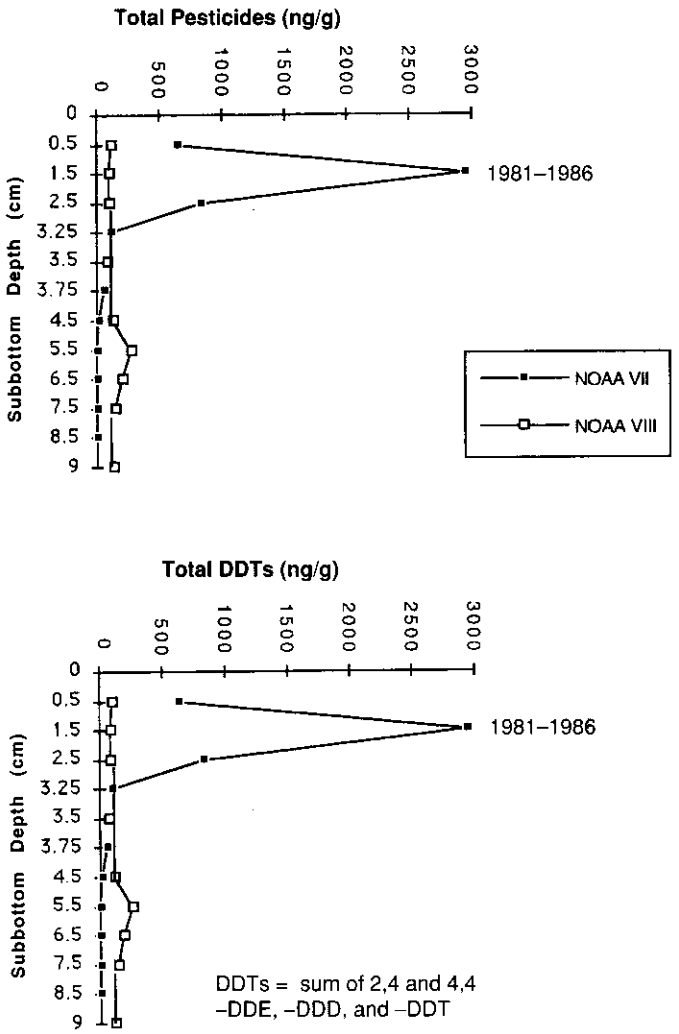

Figure 2. Historic profiles of the distribution of total chlorinated pesticides and DDTs (DDEs + DDDs + DDTs) in the sediments.

Results and Discussion

Historic profiles of chlorinated pesticides and PCBs in cores VII and VIII are presented in Figures 2 and 3. (Data and discussion for cores I–VI can be found in Venkatesan, 1994). Total pesticides in the two cores range in concentration from 2 to 3000 ng/g, and DDTs constitute from 40 to 100% of the total pesticides measured in the core sections. In general, total non-DDT pesticides in the study sites are below 20 ng/g; they appear to have been used beginning in the 1940s, their use peaked between 1948 and 1970, and they continue to be detected in surface sediments. The trend in the two cores is comparable to that observed in our prior NS&T study on cores I–VI (Figure 4).

Currently, agricultural runoff and airborne transport from land are the major sources of contamination in marine sediments. In addition, upward diffusion, resuspension, and mixing processes could bring these compounds to the sediment surface from subsurface deposits. This is most likely the major form of input of DDTs to surface sediments, since improved sewage treatment and the ban on manufacture of DDTs in the 1970s reduced the amount of DDTs entering the ocean (SCCWRP 1972–1989; Stull et al., 1986).

One striking observation is the dominant subsurface maximum of DDTs between 1981 and 1986 in core VII, which is on the southwestern region of dumpsite 2 (Figure 1). The ratios of 2,4′ and 4,4′-DDE/2,4′ and 4,4′-DDT in this horizon and the next lower section (corresponding to 1974–1981) are also the lowest (1.38 and 1.13) compared to other sections, which range from 2 to 21.73. This implies the relatively greater abundance of parent DDTs in the two sections, whereas the dominance of their metabolites in the sediments is to be expected from the source of DDTs of the recent past (pre-1970s). This anomalous distribution will be discussed later in conjunction with coprostanol and other sewage sterols.

A depth trend and an anomaly similar to that discussed above is also noticed in the distribution profile of PCBs (Figure 3). All other sections contain PCBs ranging from 4 to 1120 ng/g, comparable to that detected in cores I–VI (Venkatesan, 1994), and illustrated in Figure 5. Although point sources can be significant contributors of PCBs to the nearshore environment, nonpoint sources, such as refuse incineration and atmospheric

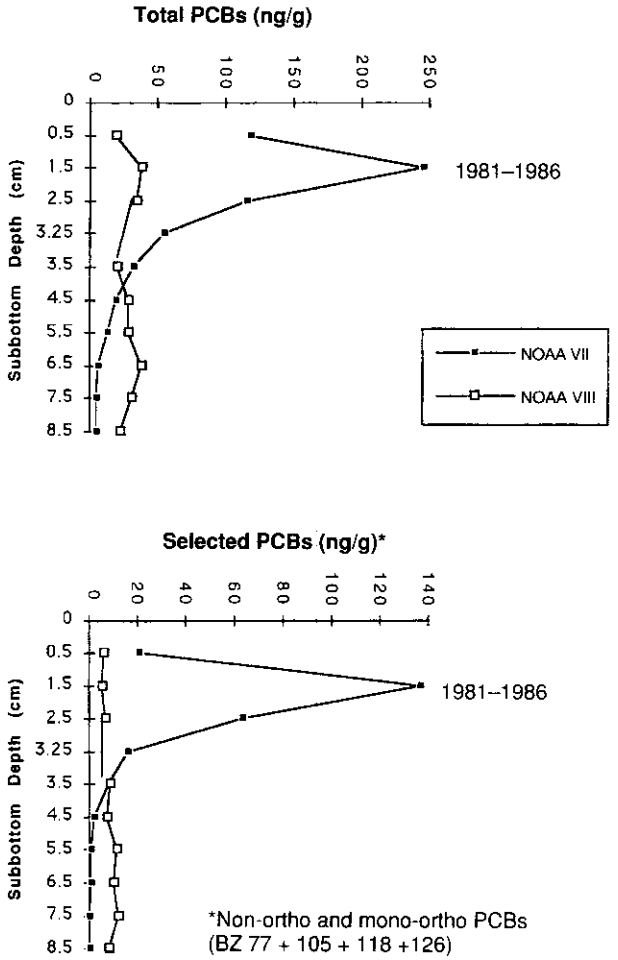

Figure 3. Historic profiles of total and selected PCBs in the sediments.

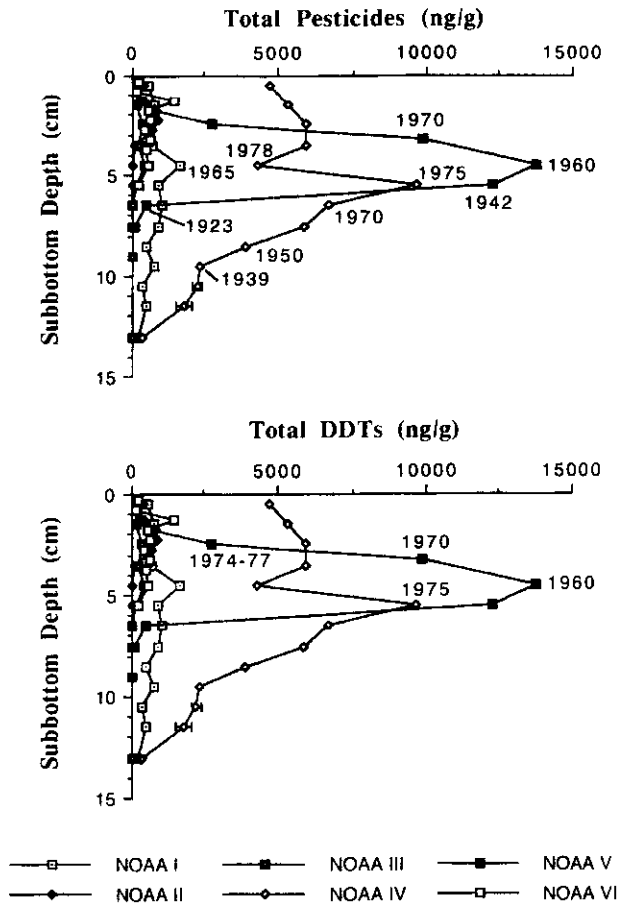

Figure 4. Historic profiles of the distribution of total chlorinated pesticides and DDTs (DDEs + DDDs + DDTs) in the sediments (from Venkatesan, 1994).

transport, runoff, etc., cannot be overlooked (Atlas et al., 1986; SCCWRP 1987; Mearns et al., 1991; Erickson 1992). However, the concordant depth trends of DDTs and PCBs suggest a common source for both suites of components, the major source generally being sewage outfalls.

Figure 6 illustrates downcore trends in the distribution of coprostanol and epicoprostanol in all eight cores, as well as trialkylamines in five cores. The coprostanol (coprostanol and epicoprostanol) content in the sediments ranges from "not detected" to 9 µg/g. Coprostanol is considered a useful tracer of fecal, and thus sewage, pollution (Walker et al., 1982; Venkatesan and Kaplan, 1990). Surface sediments from NOAA I and II contain unexpectedly comparable values (1- 2 µg/g) to those obtained from surface sections from nearby stations 40 and 102 collected in 1985 (Venkatesan and Kaplan 1990). This observation implies that the improvements introduced in Hyperion in late 1987 (i.e, the shutdown of the 7-mile pipeline) is not yet entirely reflected in the surface sediments collected in 1991. The decline in the surface section in core II corroborates this event to some extent. It is likely that the sediments in shallow waters (as in core I) is bioturbated, although Pb210 dates do not support mixing of the surface horizons.

Generally the coprostanol content is at background levels in all the SM/SP cores prior to ~1935; this is consistent with population dynamics in Southern California. A rapid increase in coprostanol content is seen to be concomitant with the rapid growth of population beginning in the 1930s (Stull and Haydock, 1988). This decade probably marks the start of the population boom in coastal urban areas (U.S. Department of Commerce, Bureau of the Census, 1982). The cores, especially those from deeper waters and anoxic regions (NOAA II, IV, and V), apparently serve as archives of wastewater inputs.

Trialkylamines (TAMs; $CH_3NR_1R_2$, where $R_1R_2 = C_nH_{2n}$, n = 16–18) are present as trace impurities in cationic surfactants, which are widely used in consumer products such as fabric softeners, anti-static agents, and cosmetic formulations. TAMs have previously been found in SM Basin sediments (Chalaux et al., 1992) and other urban coastal environments (Valls et

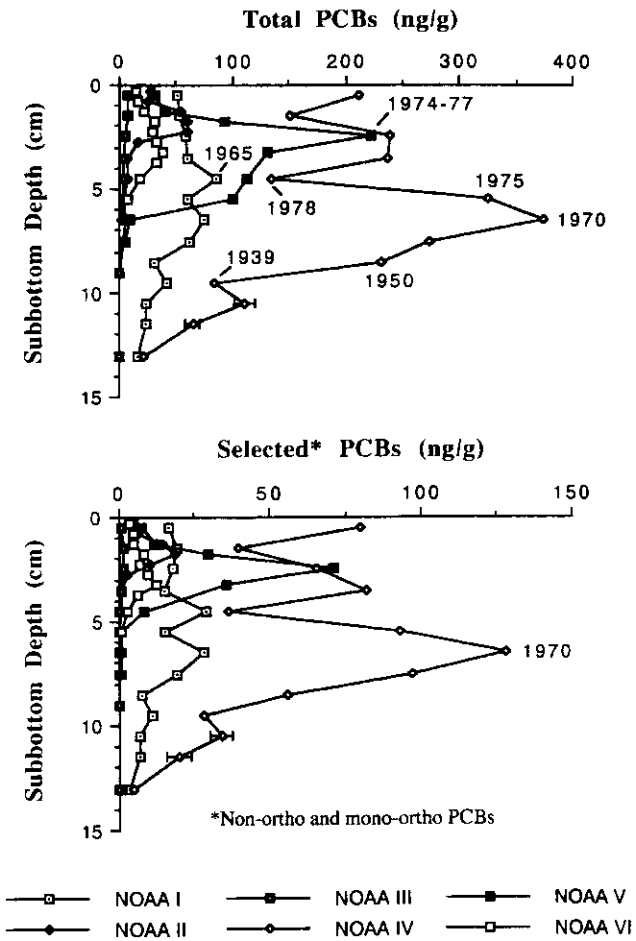

Figure 5. Historic profiles of total and selected PCBs in the sediments (from Venkatesan, 1994).

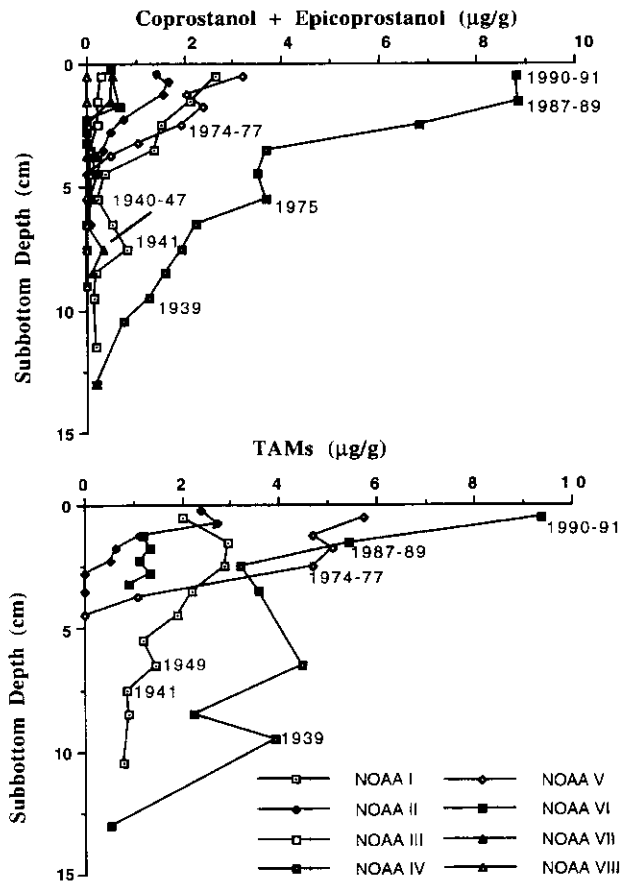

Figure 6. Selected sterols and trialkylamines from the sediments of Santa Monica and San Pedro basins.

al., 1989; Fernandez et al., 1991; Chalaux et al., 1995). As expected, TAMs and coprostanol are highest in core IV, which is from the shallow waters in the SP Basin, closer to JWPCP outfalls (Figure 1). Cores II and VI, from farther offshore, contain the least amount of the target compounds. However, it appears that the decline in concentration with increasing distance from source is more pronounced in coprostanol than in TAMs, probably because of the higher stability of TAMs. Further, their hydrophobicity may aid in the better partitioning of TAMs into sediment particles which could be laterally advected along shore, thus serving as better conservative tracers than coprostanol in tracking land-derived pollutants in offshore deeper waters.

Ratios of coprostanol/DDTs decline much more rapidly with depth (and also time) than do the absolute amounts of coprostanol alone, as is illustrated for cores I, III, IV, and V in Figure 7. The steep excursion beginning from early 1970s coincides with the change in wastewater discharge practices and the ban on DDT manufacture in the region. Apparently, content of DDTs in wastewater discharges must have been relatively higher than fecal sterols prior to 1970s to yield ratios uniformly lower than at modern times. Most of the core sections analyzed implicate wastewater as the major source of contaminants. However, some sediment intervals exhibit extremely low ratios compared to adjacent horizons in a given core, suggesting that additional unique sources of DDT must have contributed to the specific stratum as will be discussed below.

The 1981–1986 horizon (1–2 cm) in core VII exhibits a very low ratio (0.16) of coprostanol/DDTs despite the presence of significant amounts of coprostanol (480 ng/g), largely as a result of anomalously high levels of DDTs (see Figure 2). Further, the next lower section (2–3 cm, dating 1974–1981) does not contain any detectable coprostanol but contains significant amounts of DDTs at the level of 700 ng/g, suggesting a source other than municipal discharges in these two horizons. Anomalously low ratios compared to the adjacent sections were also found in the following horizons: core II: 2–2.5 cm (1966); 2.5–3 cm (1959); core III: 3–4 cm (1965); 4–5 cm (1956); 5–6 cm (1948); core V: 3–3.5 cm (1970); 4–5 cm 1960); 5–6 cm (1942). These were the same core intervals previously identified as containing organic inputs from dumping of chemical wastes (Venkatesan, 1994). This conclusion was based on the presence of relatively high amounts of DDTs (at

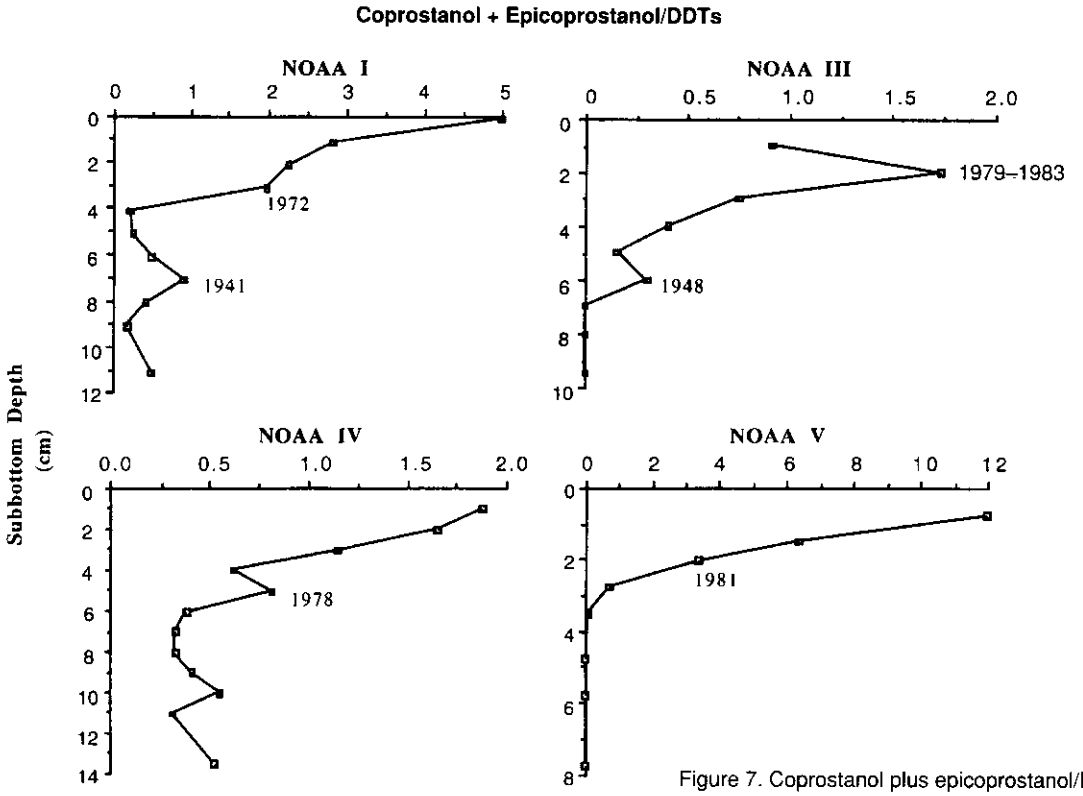

Figure 7. Coprostanol plus epicoprostanol/DDTs in sediment cores.

least 25 ng/g of 2,4′-DDT), much higher amounts of DDTs relative to DDEs (i.e., DDE/DDT ≤ 1.0), and 2,4′/4,4′-DDT ≥ 0.18, all of which suggest contributions from the caustic and/or acidic wastes characteristic of DDT manufacture (Venkatesan, 1994; Venkatesan et al., 1996). The remarkable correlation between the composition of DDTs as described above and the accompanying anomaly in the ratio of coprostanol/DDTs attests to the origin of DDTs in these sections to a major extent from dumpsites.

This conclusion is likely valid for the specific sections from core VII, due as well to its proximity to dumpsite 2 (Figure 1). Note that these two sections had a DDE/DDT ratio very close to 1.0, while all others ranged from 2 to 22. In horizons where coprostanol was not detected, but where high amounts of DDTs were measured, dumpsites in the vicinity could, therefore, be a logical source. Contaminants from the dumpsites could have diffused or advected into these horizons. Floating tar balls and DDT manufacturing wastes also are not uncommon in the general region (Venkatesan, 1994; Venkatesan et al., 1996). These could dislodge from the historic deposits and get encapsulated in modern sediment strata. This phenomenon could, explain the presence of high DDTs in the post-1970 horizons, such as in core VII.

Effluents collected in 1993 from the Hyperion and JWPCP outfalls contain comparably low levels of pesticides and PCBs (Tables 1–4). The composition of DDTs is similar, with DDEs being the significant components relative to the parent DDTs, as expected. Surface sediments near the JWPCP outfall contain DDTs that are two to three orders of magnitude greater than those from Hyperion sediments. This is apparently from the bioturbation and resuspension of historic DDTs resident in the subsurface Palos Verdes shelf sediments. PCBs are also an order of magnitude higher in these sediments than in the vicinity of the Hyperion outfalls. They were discharged via submarine sewer dischargers like the DDTs (Word and Mearns, 1979) until their manufacture and use were banned in the 1970s (Alford-Stevens, 1986). It is not surprising that the recent decline in PCBs and DDTs from JWPCP emissions parallels vertical sediment core profiles in the shelf sediments collected in the vicinity, as reported by Stull et al. (1986).

Despite a measurable increase in the flow of effluents, emission of suspended solids from Hyperion has been maintained almost at background levels since 1987 when the 7-mile sludge pipeline ceased operation (Figures 8a, b). It is also interesting to note that coprostanol levels in effluents from Hyperion and JWPCP are remarkably comparable (Tables 5, 6). With the exception of the surface horizon, dating 1988–1990, other sections in core I appear to exhibit a smooth correlation with the flow (Figures 9a, b). Similarly, suspended solids and coprostanol are also reasonably correlated except for the surface horizon. The observation that this surface layer unexpectedly contains coprostanol at the maximum levels in the core, when suspended solids in the effluents were already at low background levels, could possibly be the result of bioturbation. Although depth profiles of TAMs exhibit a slight decrease in the surface sections of cores I and II, this decline is not directly proportional to the

Table 1. Pesticides in Effluents from the Hyperion Plant and in Surface Sediments from the Vicinity

Station Sample (cm or date)	HYP EFF 10/7/93	HYP EFF 12/17/93	HYP SED 21 0–2 cm	HYP SED 21 0–2 cm
Compound (ng/g or ng/l)				
Aldrin	1.51	1.10	0.44	0.32
Alpha-Chlordane	7.45	3 03	0.17	4.88
Dieldrin	4.53	2.23	0.56	2.05
Endrin	1.50	3.03	0.27	0.46
Hexachlorobenzene	5.27	1.56	2.97	2.76
Lindane (gamma-BHC)	45.68	31.78	1.39	2.25
Heptachlor	0.13	0.60	n.d.	n.d.
Heptachlor epoxide	5.82	6.69	n.d.	n.d.
Mirex	1.05	3.25	n.d.	n.d.
Trans-Nonachlor	3.83	2.62	4.02	1.41
2,4′-DDE	2.91	2.89	32.47	17.01
4,4′-DDE	11.40	11.71	133.57	91.21
2,4′-DDD	n.d.	n.d.	12.97	9.02
4,4′-DDD	19.71	9.07	152.50	3.95
2,4′-DDT	2.78	1.96	1.01	2.09
4,4′-DDT	16.24	4.54	6.41	1.35
Total Pesticides	129.80	86.06	348.76	138.74
Total DDTs	53.03	30.17	338.93	124.62
% DDTs in Total Pesticides	40.85	35.06	97.18	89.83
% DDT (2,4′ and 4,4′) in Total DDTs	35.86	21.54	2.19	2.76
2,4′-DDT/4,4′-DDT	0.17	0.43	0.16	1.55
2,4′-and 4,4′-DDE	0.73	1.61	1.00	8.35
2,4′-and 4,4′-DDT				

Note: n.d. = not detected; below MDL

Table 2. Pesticides in Effluents from the County of Los Angeles Sanitation Districts (JWPCP) and in Surface Sediments from the Vicinity

Station Sample (cm or date)	JWPCPEFF 10/24/93	JWPCPEFF 10/27/93	x spike	PV 6C 0–2 cm	PV 8C 0–2 cm
Compound (ng/g or ng/l)			% Recovery		
Aldrin	2.38	1.59	71.09	1.74	0.67
Alpha-Chlordane	5.84	4.72	85.00	7.47	40.14
Dieldrin	3.38	1.52	94.00	70.06	23.53
Endrin	0.42	n.d.	92.00	28.38	1.04
Hexachlorobenzene	7.89	3.57	70.00	9.28	2.29
Lindane (gamma-BHC)	54.98	34.35	68.00	6.27	0.51
Heptachlor	n.d.	1.08	58.00	n.d.	0.10
Heptachlor epoxide	0.94	0.87	72.00	6.86	18.25
Mirex	4.60	2.23	87.00	1.55	18.85
Trans-Nonachlor	2.66	1.73	85.00	16.99	27.52
2,4′-DDE	3.84	3.68	91.00	1372.47	5000.03
4,4′-DDE	6.09	4.30	82.00	9316.18	33957.35
2,4′-DDD	n.d.	n.d.	103.00	198.07	1792.23
4,4′-DDD	9.35	13.32	85.00	1076.22	65044.91
2,4′-DDT	0.92	0.59	81.00	8.77	411.88
4,4′-DDT	1.45	11 30	74.00	349.43	1739 90
Total Pesticides	104.73	84.85		12469.74	108079.22
Total DDTs	21.64	33.18		12321.14	107946.32
%DDTs in Total Pesticides	20.67	39.11		98.81	99.88
%DDT (2,4′ and 4,4′) in Total DDTs	10.93	35.83		2.91	1.99
2,4′-DDT/4,4′-DDT	0.64	0.05		0.03	0.24
2,4′-and 4,4′-DDE	1.06	0.60		8.39	0.58
2,4′-and 4,4′-DDT					

Note: n.d. = not detected; below MDL

Table 3. PCBs in Effluents from Hyperion Plant and in Surface Sediments

Station Sample (cm or date)	HYP EFF 10/7/93	HYP EFF 12/17/93	HYP SED 21 0–2 cm	HYP SED 22 0–2 cm
Compound (ng/g or ng/l)				
BZ8	8.26	1.88	n.d.	0.34
BZ18	14.64	2.24	8.41	3.04
BZ28	1.83	4.82	1.85	3.24
BZ44	12.18	14.03	5.52	4.59
BZ52	9.16	16.80	6.61	4.49
BZ66	17.36	4.62	23.72	9.20
BZ77	0.98	14.07	21.72	8.09
BZ101	12.45	9.89	28.04	13.23
BZ105	3.53	2.99	13.50	10.49
BZ118	19.00	5.69	4.73	2.54
BZ126	8.26	0.93	n.d.	0.32
BZ128	0.90	0.81	11.83	4.25
BZ138	15.61	4.06	8.70	3.08
BZ153	4.76	4.24	8.21	14.02
BZ170	4.70	12.90	10.13	10.12
BZ180	4.53	25.28	4.97	2.60
BZ187	8.74	5.30	4.06	7.59
BZ195	3.97	0.24	1.61	2.27
BZ206	4.09	3.80	1.50	3.73
BZ209	5.67	8.48	1.30	3.89
Total PCBs	160.62	143.09	166.42	111.11
Non- and Mono-ortho Coplanar PCBs (Total = 77, 105, 118, and 126)	31.76	23.69	39.96	21.45
Non- and Mono-ortho Coplanar PCBs (% in Total)	19.78	16.56	24.01	19.30

Note: n.d. = not detected; below MDL

Table 4. PCBs in Effluents from the County of Los Angeles Sanitation Districts (JWPCP) and in Surface Sediments from the Vicinity

Station Sample (cm or date)	JWPCP EFF 10/24/93	JWPCP EFF 10/27/93	x spike	PV 6C 0–2 cm	PV 8C 0–2 cm
Compound (ng/g or ng/l)			% Recovery		
BZ8	4.66	25.55	43.96	13.26	9.13
BZ18	5.81	10.48	79.25	26.01	30.47
BZ28	1.97	2.39	57.36	81.72	1.29
BZ44	13.84	6.00	58.53	27.75	12.08
BZ52	4.09	3.07	64.27	51.35	28.89
BZ66	7.23	5.94	59.73	120.40	662.06
BZ77	3.82	2.96	66.45	54.92	41.55
BZ101	2.81	1.57	60.11	116.64	241.66
BZ105	2.59	1.68	61.61	84.88	30.42
BZ118	15.56	16.44	59.43	79.24	328.95
BZ126	6.19	3.10	63.76	9.77	59.26
BZ128	3.64	2.01	67.41	28.77	12.86
BZ138	9.27	14.96	69.72	70.07	300.87
BZ153	4.31	3.64	73.90	85.67	40.93
BZ170	3.74	0.83	65.40	38.54	39 24
BZ180	7.02	2.19	72.43	58.26	628.27
BZ187	6.49	3.70	69.42	40.35	32.04
BZ195	1.00	0.44	70.19	4.84	9.76
BZ206	3.35	n.d.	68.28	24.01	1.34
BZ209	17.69	7.34	62.47	6.69	36.29
Total PCBs	125.09	114.28		1023.14	2547.37
Non- and Mono-ortho Coplanar PCBs (Total = 77, 105, 118, and 126)	28.16	24.18		228.81	460.18
Non- and Mono-ortho Coplanar PCBs (% in Total)	22.51	21.16		22.36	18.06

Note: n.d. = not detected; below MDL

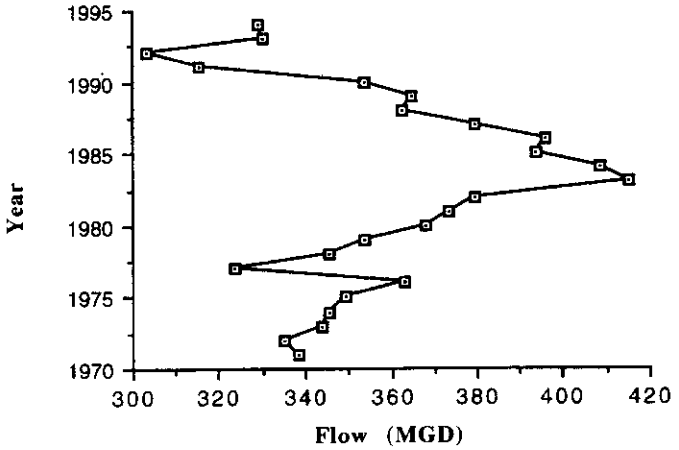

Figure 8a. Historical effluent flow data from the Hyperion plant.*
*1970–1987 = sum of Hyp 5 mi + Hyp 7 mi.

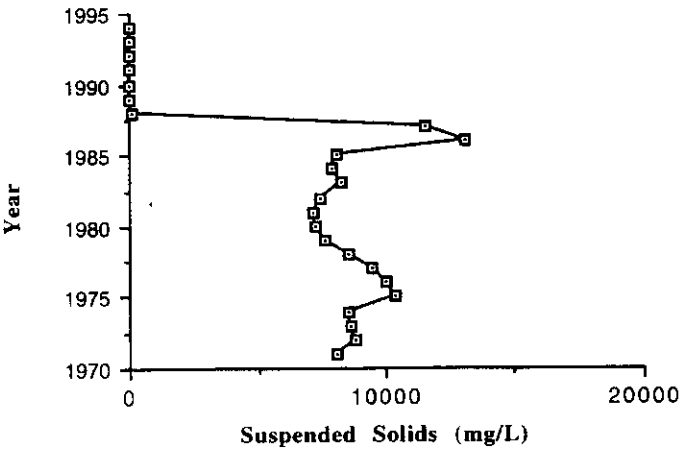

Figure 8b. Historical suspended solids data from the Hyperion plant.*
*1970–1987 = sum of Hyp 5 mi + Hyp 7 mi.

Table 5. Sterols in Effluents from the Hyperion Plant and in Surface Sediments from the Vicinity

Station Sample (cm or date)*	HYP EFF 1017/93	HYP EFF 12/17/93	HYP SED 21 0–2 cm	HYP SED 22 0–2 cm
Compound (μg/g or μg/l)				
Coprostanol	126.69	111.84	4.52	4.52
Epicoprostanol	n.d.	n.d.	0.35	0.41
Coprostanone	22.78	34 25	1.69	1.37
Cholesterol	168.15	196.29	1.90	1.54
Cholestanol	13.77	20.09	1.27	1.47
Brassicasterol	n.d.	5.69	0.63	0.62
Campesterol	10.52	13.82	1.09	n.d.
Stigmasterol	11.30	18.71	0.87	0.75
β-sitosterol	46.55	50.59	1.20	1.14
Dinosterol	n.d.	n.d.	0.61	0.69
Total Sterols	399.76	451.28	14.13	12.52
% Coprostanol + Epicoprostanol in Total Sterols	31.69	24.78	34.43	39.38
% Cholesterol in Total Sterols	42.06	43.50	13.43	12.27
Coprostanol/IEpicoprostanol	∞	∞	13.04	10.98
Coprostanol/Cholesterol	0. 75	0.57	2.38	2.94
Coprostanol + Epicoprostanol / Dinosterol	∞	∞	7.95	7.17
Coprostanol + Epicoprostanol / Total DDTs	2389.07	3707.01	14.35	39.57
Coprostanol + Epicoprostanol / Total PCBs	788.77	781.61	29.22	44.38

Note: n.d. = not detected; n.q. = too low to be quantitated because of high dilution.
*0–2 subbottom depth in cm.

Table 6. Sterols in Effluents from the County of Los Angeles Sanitation Districts (JWPCP) and in Surface Sediments from the Vicinity

Station Sample (cm or date)*	JWPCP EFF 10/24193	JWPCP EFF 10/27/93	PV 6C 0–2 cm	PV 8C 0–2 cm
Compound (μg/g or μg/l)				
Coprostanol	128.34	103.95	73.42	82.08
Epicoprostanol	1.44	n.d.	7.22	9.62
Coprostanone	28.87	19.52	23.76	30.11
Cholesterol	63.39	85.27	33.20	63.99
Cholestanol	8.93	10.94	15.04	18.06
Brassicasterol	3.14	n.d.	7.52	6.56
Campesterol	3.79	5.91	10.22	16.10
Stigmasterol	n.d.		n.d.	9.58
β-sitosterol	17.10	22.74	9.20	37.48
Dinosterol	n.d.	n.d.	3.90	3.45
Total Sterols	255.01	248.32	183.50	277.03
% Coprostanol + Epicoprostanol in Total Sterols	50.89	41.86	43.95	33.10
% Cholesterol in Total Sterols	24.86	34.34	18.09	23.10
Coprostano/Epicoprostanol	∞	∞	10.16	8.53
Coprostanol/Cholesterol	2.02	1.22	2.21	1.28
Coprostanol + Epicoprostanol / Dinosterol	∞	∞	20.65	26.61
Coprostanol + Epicoprostanol / Total DDTs	5997.54	3132.83	6.55	0.85
Coprostanol + Epicoprostanol / Total PCBs	1037.55	909.59	78.82	36.00

Note: n.d. = not detected; n.q. = too low to be quantitated because of high dilution.
*0–2 subbottom depth in cm.

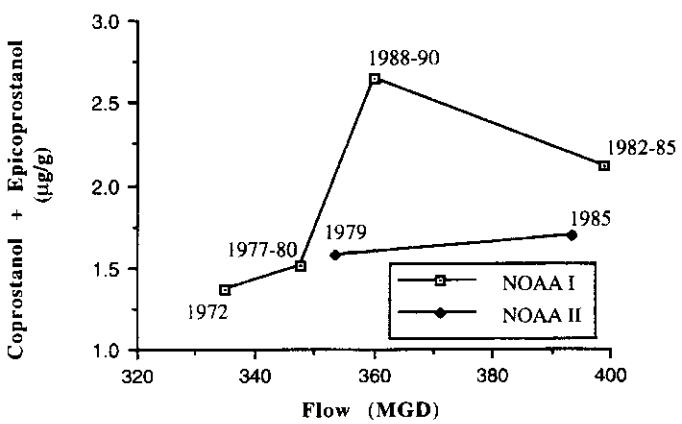

Figure 9a. Coprostanol vs. flow of Hyperion effluent.

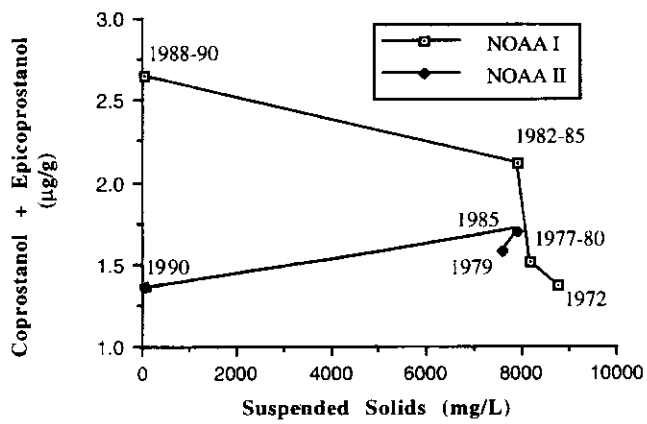

Figure 9b. Coprostanol vs. suspended solids from Hyperion effluent.

dramatic reduction in the output of solids from the 7-mile pipe, again implying bioturbation.

Effluent flow as well as suspended-solids emission from the JWPCP outfall have stabilized to constant background levels since 1985 (Figure 10). The surface horizon from core VI represented only the period prior to 1987 and thus does not reflect this trend (Figure 6). More recent sediments from core IV, closer to the outfalls, do not exhibit a strong correlation of coprostanol with flow rates (Figure 11a). However, following a steep rise from 1982 to 1986, coprostanol content appears to have stabilized from 1987 to 1991, concomitant with the near-constant levels of suspended solids (Figure 11b). This probably implies the association of coprostanol with particulate matter which could be advected offshore. In contrast, the abundance of TAMs in this core continues to increase from 1987 to 1991, possibly implying increased use by the urban population and subsequent output of TAMs in the wastewater. However, core V, from the deeper basin, exhibits almost superimposable profiles of coprostanol and TAMs content, with the topmost section (0–1 cm, ca. 1989–1991) containing the maximum of both components. (These two components were analyzed by two different laboratories: coprostanol at UCLA and TAMs at CSIC in Spain). Core V is from the anoxic basin and is devoid of measurable bioturbation, and thus should serve as an archive of wastewater history. This observation is also confirmed by core II from the deeper SM basin, where the historic trends are similarly better preserved.

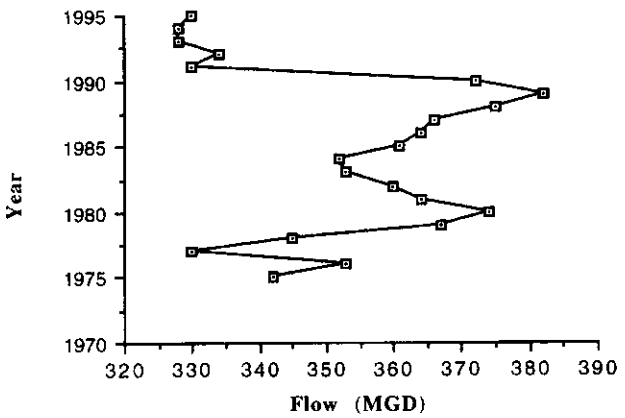

Figure 10a. Historical effluent flow data from the JWPCP outfall.

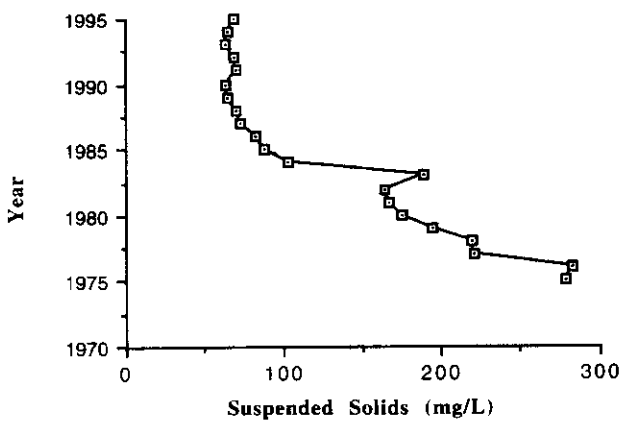

Figure 10b. Historical suspended solids data from the JWPCP outfall.

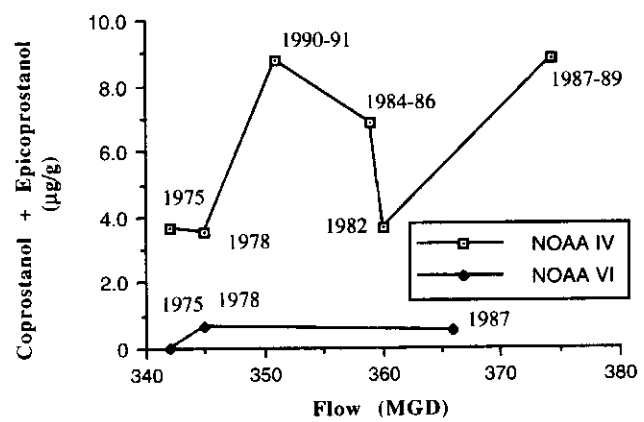

Figure 11a. Coprostanol vs. flow of JWPCP effluent.

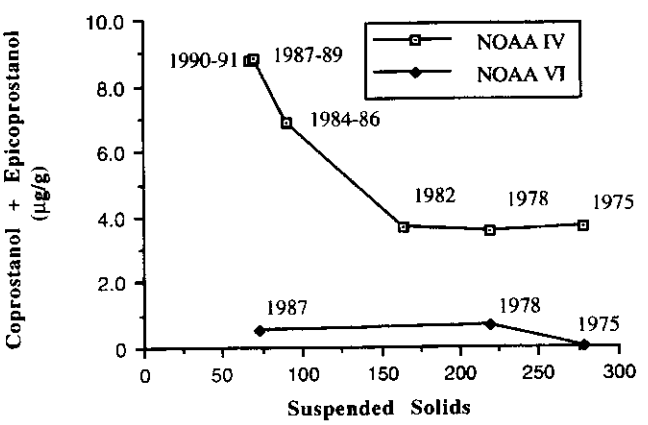

Figure 11b. Coprostanol vs. suspended solids from JWPCP effluent.

Conclusions

The data generally document the impact of the change in wastewater technology, which was the first major objective of this project. Core II from the SM Basin and core V from the SP Basin reflect the history of wastewater inputs in the region better than other cores. The surface horizons (including those from other cores) to a limited extent reflect recent improvements in wastewater technology, which have helped curb the output of suspended solids, even though the volume of effluent flow has not declined over the last decade. In particular, there is a reflection in the top layer of core II of the cessation of the Hyperion 7-mile sludge line. The efficient removal of solids from JWPCP effluent is registered by core V in the "reluctant" increase of the target tracers over the last decade. Interestingly, the decline in solids output from the waste dischargers may delay the formation of a protective layer of relatively clean sediments over the horizons containing toxic DDTs wastes of the past!

Data on DDTs from our prior NOAA, NS&T project, which identified that certain sedimentary strata contain contaminants from nearby dumpsites, are well corroborated by the present study on coprostanols from the same sediment horizons. The specific chemical parameters developed from the relative amounts of various contaminants measured here helps differentiate contaminant inputs from wastewater from those originating in the offshore dumpsites, thereby accomplishing the second major objective of this project.

The trialkylamines appear to be good conservative tracers of sewage inputs in the deeper waters of the basins investigated.

Acknowledgments

I thank Rosario P. deLeon, Xinwei Ouyong, Sharif Sawires, and Greg Vaughn for technical assistance; Ed Ruth for GC/MS and GC/ECD analysis; M. Baskaran for Pb-210 analysis of selected sections from cores VII and VIII; Jan Stull, Bill Holtz, John Shisko, and John Dorsey for effluent and sediment samples from the vicinity from JWPCP and Hyperion outfalls and for pertinent data; and Azra Khan of SCCWRP for relevant data on effluents. Sediment cores I–VI were collected and sterol fractions isolated as part of NOAA, NS&T Program.

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County of Los Angeles Sanitation Districts
City of Los Angeles Sanitation Districts
Environmental Chemistry Department, CID, CSIC, Barcelona, Spain

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M.I. Venkatesan, C. Maldonado, and J.M. Bayona. Sewage markers in the sediments of Santa Monica/San Pedro Basins, California. Presented at the Division of Environmental Chemistry, American Chemical Society Meetings, Orlando, Florida, August 1996. Abstract.

Invasion of California Estuaries by the Nonindigenous Green Crab *Carcinus maenas*: Assessment of Impact and Geographic Spread

University of California, Santa Barbara
R/C-22-16PD
1993–94

Armand M. Kuris and Kevin D. Lafferty

The goals of this project were to (1) assess the ability of the introduced green crab *Carcinus maenas* to prey on some important California invertebrate species, (2) gauge the geographical spread of the crab in California, (3) begin a preliminary examination for parasites, and (4) gather information on the size distribution of the crabs in South San Francisco Bay. Goals (1) and (2) were conducted in collaboration with E.D. Grosholz and G.M. Ruiz.

Collections throughout the San Francisco Bay area have indicated the abundant presence of *C. maenas* in the South Bay (Redwood Shores Lagoon), the East Bay (Berkeley Yacht Harbor), the North Bay (Tiburon), and the lower salinity waters of the Carquinez Straits and San Pablo Bay. A large set of crabs has been detected in Drakes Estero, Tomales Bay, and Bodega Harbor to the north of San Francisco Bay. Crabs were also present in Bolinas Lagoon. Extensive searches have not recovered crabs from Salt Point, the mouths of the Gualala and Noyo rivers, or Humboldt Bay to the north until the spring of 1995 when green crabs were collected in Humboldt Bay. Searches of Half Moon Bay, Elkhorn Slough, and Morro Bay to the south produced no findings until the winter of 1994–1995 when a single green crab was recovered from Elkhorn Slough.

The spread of green crabs from San Francisco Bay to Tomales Bay and Bodega Harbor to the north is historically noteworthy. Most of the 250 or more introduced species in San Francisco Bay have not yet spread beyond the confines of the bay (Carlton, 1979). For this new animal to do so with massive sets of postlarval crabs is remarkable. Even the phenomenally abundant introduced Asian clam, *Potamocorbula amurensis* (more than 10,000/m^2), has not expanded its geographical range beyond San Francisco Bay (Carlton et al., 1990).

In predation experiments, prey items of various sizes were offered to individual male crabs. The number of prey killed were recorded daily. Consumed items were not replaced so that size preferences could be assessed. Crabs had carapaces 50–75 mm wide. The crabs were highly effective predators on mussels, *Mytilus galloprovincialis*, less than 60 mm and on red abalone, *Haliotis rufescens*, less than 40 mm. Only a few sea urchins, *Strongylocentrotus purpuratus*, were consumed, most less than 30 mm in diameter. Trials with Dungeness crabs were unsuccessful, because we were unable to obtain crabs smaller than 60 mm. However, native grapsid crabs less than 60 mm were consumed at a high rate. Likewise, trials with oysters were unsuccessful, because none were available smaller than 50 mm. For the three types of prey consumed, predation rates were high. For example, mussels were eaten at a rate of 3.4 per day when preferred sizes were available. Overall, these results suggest a potentially significant impact on species of great interest for mariculture in openwater facilities. Prey items will ultimately grow to a size at which predation will be negligible, but many young prey will be lost to these predators.

The results of the predation experiments are generally consistent with those studies of the food habits of green crab that were conducted elsewhere (Ropes, 1968; Elner, 1981). As Le Roux et al. (1990) reported, sea urchins were rejected. The ability of green crabs to feed on abalone is a potentially serious additional impact on the Pacific coast of North America. As elsewhere (Ropes, 1968; Jensen and Jensen, 1985; Cohen et al., 1995; Grosholz and Ruiz, 1995), a strong adverse impact on shallow-water commercial invertebrate fisheries and on mariculture cages and ropes is to be expected.

Fifty-five adult female green crabs 44–62 mm in diameter were dissected and carefully examined for parasites. No parasites were recovered, a remarkable result, because this species is heavily parasitized in Europe (Crothers, 1968).

Crabs trapped in San Francisco Bay over the winter months were large (most males were 50–80 mm wide; females 48–62 mm). These sizes are in the largest fraction of crabs from European samples (Crothers, 1967, 1968; McGaw et al., 1992). Inadequate sampling of small crabs prevents a conclusion about growth of crabs in San Francisco Bay, but these observations are consistent with a strong growth performance for these crabs. Also, these crabs may have a higher survivorship than that reported for European populations. A study of molting frequency and molt increment should enable these alternatives to be distinguished. In either case the available evidence supports the hypothesis that these introduced crabs may be experiencing a form of ecological release in San Francisco Bay.

Cooperating Organizations

The Cultured Abalone, Inc.
Sportsman's Bait

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The Effect of Trampling on Marine Rocky Shores in Southern California

Lewis & Clark College
R/C-22-17PD
1993–94

Deborah M. Brosnan, John Elliott, and Ingri Quon

Rocky shores are important recreational and educational resources. Worldwide these areas are coming under increasing pressure, and trampling by humans is becoming a major concern in the United States (Zedler, 1976, 1978; Beauchamp and Gowing, 1982; Brosnan and Crumrine, 1992, 1994; Brosnan, 1993), New Zealand (Cole et al., 1990), Australia (Yapp, 1986; Liddle and Kay, 1987; Kay and Liddle, 1989; Underwood and Kennelly, 1990; Kingsford et al., 1991; Liddle, 1991; Povey and Keough, 1991), and Britain (Boalche et al., 1974). This trend is likely to rise as increases in tourism and population bring more and more visitors to the shore.

Previous work established that trampling affects intertidal organisms. These effects can be dramatic (Boalche et al., 1974; Brosnan and Crumrine, 1992, 1994; Brosnan, 1993). Community structure can be altered, biodiversity reduced, and the whole appearance of the shore changed (Bolache et al., 1974; Povey and Keough, 1991; Brosnan and Crumrine, 1992, 1994). In an observational and experimental study at Yaquina Head, a heavily used marine garden in Oregon, Brosnan and Crumrine (1992) found the lowest diversity in the most heavily trampled parts of the shore. Diversity was highest in areas with limited access and fewer visitors. Diversity increased when trampling was stopped for 5 months and subsequently declined to pretrampling levels, when trampling restarted. Not all species are equally affected by trampling. Morphology, growth form, and means of attachment to the substrate can influence susceptibility. Foliose algae are more readily removed than crusts or algal turf (Povey and Keough, 1991; Brosnan and Crumrine, 1992, 1994), and foliose species are often rare or absent from trampled shores (Boalche et al., 1974; Beauchamp and Gowing, 1982; Povey and Keough, 1991; Brosnan and Crumrine, 1992, 1994). In an experimental study at two sites on the Oregon coast, Brosnan and Crumrine (1992, 1994) and Brosnan (1993) found that when foliose algae were trampled, the plants (mainly *Iridaea, Pelvetiopsis*, and *Gigartina*) declined rapidly from 80% cover to less than 20% cover.

Rocky shores in southern California are heavily affected by humans (Beauchamp and Gowing, 1982; U.S. National Park, 1992; Foster, personal communication, 1993). Yet, few studies (Zedler 1976, 1978; Beauchamp and Gowing, 1982; Ghazanshahi et al., 1983) have been carried out, and no experimental studies have been reported, on how trampling affects the Californian marine ecosystem. Preliminary observations (Brosnan, 1993) suggest that species composition may be significantly affected by trampling. For example, Brosnan (1993) and Foster (personal communication, 1993) noted that low-relief corraline algae dominate many heavily visited sites in California. This is unusual; normally corraline algae occupy less than 10% of total space. They are inferior competitors and are often confined to tide pools. Corraline algae are prostrate algae, which are flat against the substrate, and are generally less than 2 mm thick. Consequently, these species may be resistant to trampling. Other algae may also be affected. A larger percentage of the *Endocladia* population is present as turf rather than in upright form on heavily visited shores in the National Marine Sanctuary in Monterey (Brosnan, 1993, personal observation). Foliose algae, which dominate most rocky shores on the Pacific coast of the United States are less common on trampled sites in California (Brosnan, unpublished data).

Methods

Study sites. Trampling experiments were conducted at two mid-intertidal sites on the southern California coast near La Jolla: Horseshoe and South Bird Rock. Observations of human activities were made at Horseshoe. Both sites consist of sandstone platforms. The biological community differed between sites, and experiments were set up to look at the effect of human trampling in these two communities.

The site at Horseshoe consists of mostly low-relief algal turf of which *Gigartina* species are dominant. Seasonal sedimentation occurs at this site. Visitors can easily reach the shore here, and parking is available within 5 m of the shore. The area is heavily used by recreational visitors and is also occasionally used as a mountain-bike trail.

The densities of algae and invertebrate are higher at Bird Rock than at Horseshoe. Access to South Bird Rock by humans is relatively restricted because of private development and high cliffs. However, this shore is used by visitors, for recreation and education, and surfers cross the intertidal area en route to the ocean. South of the main access are rocky outcrops dominated by *Pelvetia*. We set up experiments here because fewer visitors use this part of the shore.

Trampling method. At each site, we set up a randomized block design to test the effect of trampling on marine assemblages. Four blocks, each consisting of two treatments, trampled and control (untrampled) were set up at each shore. We trampled the "trampled" plots by walking across each plot 50

times on one day every month. The experiment was carried out from June to September 1993.

Data Collection and Statistical Analysis

Data were collected on the percentage of cover of primary space, bare space, and canopy species. The percentage of cover of each species was estimated by placing a clear vinyl sheet marked with 100 randomly spaced dots directly over the plot. The number of dots directly over a species was counted. For algae, the primary percentage cover was defined as the percentage of the substrate on which a species was directly attached. Algal canopy was defined as the percentage of the rock surface that a nonencrusting alga covered, although it might not be attached to that particular point. Data were arcsine transformed to reduce heteroscedasticity and analyzed by using RMANOVA.

Results

At Horseshoe trampling had little effect on cover of the red algal mat. *Gigartina* species continued to dominate the algal assemblage in control and trampled sites. A difference in cover between trampled and control sites was detected only once.

At Bird Rock, trampling reduced abundance of the large foliose alga *Pelvetia*. In trampled plots, the percentage of cover declined from 90% to 54% between June and August 1994. Cover remained unchanged in control (untrampled) plots. Trampling did not decrease canopy cover of algal turf (*Gigartina* species). Trampling also reduced the amount of primary cover. Most of the decline was due to a loss of both upright corraline algae and *Pelvetia* holdfasts.

Discussion

In previous studies on the Oregon coast, Brosnan and Crumrine (1992, 1994) found that the impact of visitors changed the intertidal community from one dominated by mussels and foliose algae to a low-diversity algal-turf assemblage. They predicted that large foliose algae, which are attached by single holdfasts, would be susceptible to trampling and that algal turf would be resistant. This study supports these predictions and the results of previous studies (e.g., Zedler, 1976, 1978; Beauchamp and Gowing, 1982). In our study, trampling had little effect on the turf-dominated assemblage at Horseshoe. By contrast, trampling reduced the amount of upright algae at Bird Rock.

Interestingly, at Bird Rock *Pelvetia* is abundant only in areas that receive fewer visitors. In addition, many of these areas are slightly offshore and are quickly cut off by incoming tides, making them relatively inaccessible for much of the tidal cycle.

Large foliose algae were absent from Horseshoe. This may have been due to differences in wave action (Horseshoe is less exposed than Bird Rock), sedimentation (sediment depth at Horseshoe can exceed 2 cm), or substrate suitability. However, at other heavily visited sites, *Pelvetia* and other foliose species were found only on the sides of rocks (unpublished data). The surfaces of the rocks were bare or covered by algal turf.

In conclusion, trampling reduces the abundance of large foliose algae. The shores in La Jolla have been heavily affected by visitors since the late 1800s (La Jolla Historical Society, personal communication), and it is interesting to speculate how much of current diversity of the shores is the result of human impact.

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Cooperating Organizations

La Jolla Historical Society
Sustainable Ecosystems
San Diego Historical Society

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Acousto-Optic Studies of La Jolla Canyon

Scripps Institution of Oceanography
R/OE-22-13PD
1993

Jules S. Jaffe

This proposal requested funds to perform a variety of remote sensing activities in the La Jolla Canyon, an area offshore from the Scripps Institution of Oceanography, in La Jolla, California. The basic plan was to take our three-dimensional sonar imaging system, FTV, on the research vessel *Robert Gordon Sproul* for several days to test the system's capability in several ways. The cruise was extremely successful, and we completed most of the tasks that we had outlined in our original proposal.

The sonar was deployed on a remotely operated vessel (ROV), that the Marine Physical Laboratory owns and operates. An instrument that measures conductivity, temperature, and depth, and a fluorometer were also deployed on the ROV. A basic goal of the experiments, was to see if we could track individual zooplankters in the water column for periods as long as possible. Over a 2-day period, the *Sproul* was deployed over the La Jolla Canyon, and the ROV was used in the water for periods as long as 8 hours. The system was used in several different ways. In one case the system was kept at a fixed depth and was used to monitor the rate of flux of animals that were migrating vertically. The system was also deployed in a drifting mode in which it was used to follow particles with almost Lagrangian performance. Additionally, the video imaging system was used on the ROV to image the animals that were also in the field of view of the camera system

The cruise took place July 9–11, 1993. Over the next several months, we analyzed the data from the cruise and basically validated that the technology performed as desired. Using the positional data from the system, we have developed algorithms that depict the power spectra of the spatial distribution of the small scatterers over volumes as small as 4–5 m^3. Relative changes in the animal distributions as a function of time of day were analyzed. The primary advantage of this system, in this regard, is the almost instantaneous observation of three-dimensional animal distributions that the system produces.

In addition, Sea Grant trainee Duncan McGehee, has developed algorithms that enable us to follow the trajectories of particles within the field of view of the sonar. He has been able to compute the trajectories of six targets that were simultaneously within the field of view of the sonar. We think that this is a major achievement for our group and that the funding of this research by this rapid response initiative has made this possible.

Genetic Diversity Within and Between Laboratory-Reared and Natural Populations of Abalone (*Haliotis* spp.)

California State University, Northridge
R/NP-1-21S
1992–93

Robert C. Carpenter and Kenneth C. Jones

Abalone (*Haliotis* spp.) are an important component of nearshore marine communities in California. In many environments, harvesting has led to drastic declines in the abundance of abalone that have necessitated the imposition of strict harvesting guidelines (Tegner et al., 1989). As a result of their decline, abalone have been the focus of intense management efforts for protection and maintenance of a sustainable harvest from existing populations.

Abalone have also been the target for mariculture efforts designed to rear the animals to a minimum size, either for human consumption or for outplanting into natural areas depleted of this resource. Providing more effective management and obtaining maximal yields of laboratory-reared stocks require a thorough understanding of the population biology of the species.

A critical aspect of the population biology of abalone is the spatial extent of the dispersal of offspring, which determines, in part, the genetic composition of the population. The longevity of the dispersive larval stage varies from 3 to 5 days. Although many investigators have assumed long-distance dispersal of larvae during this planktonic period, recent evidence suggests much more limited dispersal (Prince et al., 1987, 1988; Brown, 1991). Limited dispersal increases the likelihood of inbreeding, which, in turn, will result in a much lower level of genetic diversity than exists in outbred populations.

Previous studies (Prince et al., 1987, 1988) used correlations between the time of spawning and subsequent settlement or recruitment to infer the distance larvae were dispersed. Brown (1991) used isozyme analysis to investigate gene flow between populations of abalone in Australia and concluded that dispersal occurred over spatial scales greater than 100 km but that some populations were genetically isolated over much shorter distances.

Molecular approaches to population-level questions have become increasingly common as techniques have been developed to isolate and amplify small quantities of DNA. Restriction-fragment length polymorphism (RFLP) analysis has been used to estimate the spatial extents of populations of plant (Schaal et al., 1991; Fain et al., 1992) and animal (Karl and Avise, 1992) species. This approach provides the resolution necessary to identify genetic markers to delimit populations.

Our initial goal was to determine if tissue suitable as a source of DNA could be obtained without killing the animal.The decline of many abalone populations makes a nonlethal method of sampling desirable. To further reduce damage to the animal, we used a polymerase chain reaction (PCR) to amplify DNA from minute amounts of tissue. We were successful in amplifying DNA from gill and mantle tissue that was obtained without sacrificing the animals. After amplification, the amount of DNA obtained from less than 100 mg of tissue was sufficient for RFLP analyses.

For RFLP analyses, we focused on the internal transcribed spacer (ITS) sequences of the ribosomal DNA (rDNA) repeat unit. These regions lie on either side of the 5.8S rRNA coding sequence and between the 18S and 28S rRNA coding sequences. The ITS regions are known to be variable, whereas the adjacent coding regions, in contrast, contain sequences that are highly conserved over a wide taxonomic range (Hillis and Dixon, 1991). Because of this arrangement, PCR primers can be synthesized in the absence of sequence information obtained specifically from abalone. The primers can then be used to amplify the ITS regions and the included 5.8S sequence. Because of the relatively nonconserved nature of the ITS regions, treatment of the PCR products with restriction endonucleases produces polymorphic fragments at a relatively high frequency. Using primers consisting of sequences originally obtained from mouse and frog, we were able to produce a single PCR product approximately 1100 base pairs long. This product was used for RFLP analysis. Variations in the length of the fragments obtained from abalone from different populations reflect variations in the presence or absence of restriction sites recognized by the enzymes used in the analysis. Using this approach, we have detected genetic differences among the animals obtained from several populations of abalone.

Materials and Methods

For DNA isolation, gill and epipodial tissues were obtained from red and black abalone (*Haliotis rufescens* and *Haliotis cracherodii)* provided by McCormick & Associates mariculture facility. Genomic DNA was isolated by using a tissue-lysis protocol (Higuchi, 1989). This procedure can be used as a nonlethal sampling method because it requires only about 70–100 mg of epipodial tissue.

Samples of red abalone were obtained from four natural populations and three from laboratory-reared populations. Fifteen individuals each were collected from Santa Rosa Island, San Nicholas Island, and Fort Bragg. Only four individuals were available and sampled at Santa Cruz Island. In addition, three animals from separately breeding populations were used from the mariculture facility.

The ITS regions between the 18S and 28S subunits of the DNA repeat unit of abalone genomic DNA were amplified by using PCR (Hillis and Dixon, 1991). The PCR products were purified and aliquots of each product were treated with each of the following seven restriction enzymes: Dpn II, PuvII, HhaI, HinfI, MspI, TaqaI, and NciI. Restriction fragments were separated by gel electrophoresis, stained with ethidium bromide, and examined for polymorphism.

Results

Eighteen distinct sets of restriction fragments were identified. The enzymes HhaI and PuvII did not reveal any polymorphism among sampled populations. Table 1 summarizes the results obtained with the remaining five enzymes.

The enzyme HinfI produced two sets of fragments. Three haplotypes were identified with MspI. Results of treatment with these two enzymes indicated that the tested populations of abalone are genetically similar, even though some polymorphic individuals exist in each population.

The enzyme NciI revealed three haplotypes. The halotype found in the three laboratory specimens was not found in the natural populations. NciI did not reveal genetic differences among the natural populations.

The enzyme DpnII revealed four haplotypes. The frequency of these four varied between and among populations. One of them had a particularly high frequency in the San Nicholas sample.

Three haplotypes were revealed by TaqaI. Results of treatment with TaqaI suggested that the San Nicholas population can be distinguished from the others by the frequency of one haplotype.

Table 1. Number of Red Abalone Containing Fragment Sets Detected by Restriction Digestion of rDNA Internal Transcribed Spaces Sequences

Restriction Enzyme	Population	Fragment Sets			
		A	B	C	D
DpnII	FB	7	7	1	0
	SR	4	7	3	1
	SC	0	4	0	0
	SN	4	11	0	0
	F	0	0	2	1
HinfI	FB	14	1	0	0
	SR	12	3	0	0
	SC	4	0	0	0
	SN	14	1	0	0
	F	2	1	0	0
NciI	FB	14	0	1	0
	SR	13	0	2	0
	SC	4	0	0	0
	SN	15	0	0	0
	F	0	3	0	0
TaqaI	FB	9	3	3	0
	SR	12	0	3	0
	SC	3	0	1	0
	SN	1	2	12	0
	F	0	3	0	0
MspI	FB	10	3	2	0
	SR	13	1	1	0
	SC	4	0	0	0
	SN	9	4	2	0
	F	2	1	0	0

FB = Fort Bragg, SR = Santa Rosa, SC = Santa Cruz, SN = San Nicholas, F = McCormick & Associates.

Discussion

An important aspect of the population biology of any species is the distance over which offspring are dispersed. The spatial scale of dispersal affects the balance of local selective pressures due to environmental heterogeneity and the ability of the species to "respond" to such heterogeneity. The swamping effect is due to the influx of offspring from environments with different selective pressures. The extent of dispersal and gene flow between populations of a species determines the degree of genetic similarity between individuals from different populations and the amount of genetic diversity within a single population. If dispersal is limited, populations can become isolated and then diverge genetically through the processes of selection and genetic drift. Such isolated populations often have reduced genetic diversity because of inbreeding. Determining the spatial scale of dispersal and gene flow not only provides a better understanding of the population biology of a species but also is important for the effective management of commercially harvested resources.

The dispersability of a species can be correlated with the type of reproduction and the propagules produced by that species. Marine organisms produce juveniles that range from brooded larvae that are dispersed only a short distance from the parent to teleplanic larvae that feed in the plankton and are dispersed over thousands of kilometers. Reduced amounts of gene flow between populations would be predicted for the former, whereas a high degree of genetic mixing, and therefore similarity, would be predicted for the latter. Abalone produce planktonic larvae that are relatively large and that spend a short time (3–5 days) in the plankton. Depending on the point of release relative to ocean currents, the spatial scale of dispersal would be predicted to be relatively short. Although investigators previously have assumed long-distance dispersal of abalone larvae because of the larvae's planktonic nature, recent studies have suggested that the scale of dispersal is much more limited (Prince et al., 1987, 1988; Brown, 1991).

On the basis of our preliminary data from RFLP analysis of the ITS region of rDNA, we tentatively conclude that populations of red abalone are genetically distinct over spatial scales of more than 100 km. Haplotype frequencies of red abalone from San Nicholas Island are distinct from those of all other sampled populations. Differences between other populations are present, but are not as convincing. Because of the preliminary nature of this study and the small number of animals analyzed from each population, statistical analyses of these data are inappropriate.

These data suggest that abalone larvae have an intermediate dispersal capability and, as a result, will have a degree of genetic structuring among subpopulations that reflects the spatial scale and frequency of dispersal. These data further suggest a difference between mainland populations of abalone and those on offshore islands. San Nicholas Island is the farthest of the California Channel Islands from the mainland, and populations of abalone there may be separated from populations of other Channel Islands and the mainland by the California Current. As a result, because of genetic drift or selection or both, they have diverged genetically. Populations of abalone along the mainland may not be as isolated because of the "stepping-stone" effect of dispersal along the coast, which may result in sufficient gene flow to maintain genetic similarity over distances that are much longer than the dispersal of abalone larvae. We do not have data to support this hypothesis.

From our data, we conclude that populations of red abalone from San Nicholas are genetically distinct from the other populations sampled. The relative genetic similarity of the remaining populations has two possible explanations: (1) populations are genetically uniform because of frequent gene flow between them, or (2) populations are genetically distinct, but because of the relatively low levels of genetic variation within the ITS region of rDNA of abalone, we can cannot detect the degree of genetic differentiation between populations by using this technique.

This conclusion leads to a testable hypothesis that we are proceeding to address by using a higher resolution genetic technique involving short tandem repeat (STR) sequences. This technique has been used successfully with other taxa to detect genetic variation at the population level and should lead to a more definitive conclusion about the spatial scale of dispersal and gene flow between populations of abalone in California coastal habitats.

Selection of genetic markers associated with desirable growth characteristics or other phenotypic characters is proceeding. For this purpose we are attempting to detect STR loci. These loci are used as markers of quantitative trait loci in various species. The STRs are most likely distributed throughout the abalone genome. We have obtained and labeled STR sequences, and we are testing the abalone genome to determine if these sequences are present. The likelihood is high, as STR loci have been reported in all eukaryotes studied, except yeast. If present, STR loci can also be used for population studies that would afford higher resolution than the rDNA ITS regions.

Cooperating Organizations

California Abalone Association, Santa Barbara

California Department of Fish and Game, Long Beach and Fort Bragg

Channel Islands National Park, Ventura, California

McCormick & Associates, Oxnard, California

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Erosion of Cordgass at Kendall-Frost Mission Bay Reserve

Scripps Institution of Oceanography, UCSD
R/C-22-21PD
1993–94

Paul K. Dayton and Lisa A. Levin

The Kendall-Frost Mission Bay Reserve is a small remnant of the original large wetland that once covered Mission Bay near San Diego. This marsh is the only remaining marsh of its type in Southern California to show a full range of estuarine habitats from upland, transition zone, high marsh to mid-marsh, low marsh, mudflat, and eelgrass. Cordgrass (*Spartina foliosa*) makes up the low marsh. The cordgrass is the critical habitat of the endangered California light-footed clapper rail, which builds its floating nest among the stems of the grass. Over the past 6 years, an alarming decline has occurred in this important habitat. As the *Spartina* disappears, the low marsh collapses into the mudflat, and the reserve loses an entire wetland habitat, perhaps the most threatened habitat on the west coast of the United States.

Monitoring since 1989 shows the loss of as much as 5 m of this habitat along the southern end of the marsh and massive deterioration of the cordgrass over the entire marsh. Erosion is attributed to jet skis and the wake from motor boats, the loss of cordgrass, and intrusion of beach sand. The deterioration of the cordgrass is hypothesized to result from lack of fresh water and nutrients (Zedler and Powell, 1993). Marshes are commonly known to be nitrogen limited (Mitsch and Gosselink, 1986). The Kendall-Frost marsh has no source of fresh water. Rose Creek, a freshwater creek that once flowed through the marsh, is now diverted into a cement channel that flows directly into a dredged portion of the bay. This, combined with the drought conditions experienced throughout the late 1980s and early 1990s, could have stressed the plants and started their decline.

A pilot experiment was started to evaluate the effects of fresh water and fertilizer on cordgrass biomass. The treatments consisted of fresh water only, fertilizer only, both fresh water and fertilizer, and no treatment (controls). Results showed that a combination of fresh water and fertilizer resulted in a significantly higher above-ground biomass (Figure 1). The areas treated with both fertilizer and fresh water had significantly higher biomass than the control areas. These results are preliminary, but such experiments are needed to learn the causes of the decline. Meanwhile, treatments such as these will be needed as a life-support system to preserve the cordgrass habitat until a more permanent solution is found. Work continues to detect thresholds and to enlarge the spatial area of the experiments so that the deterioration of the cordgrass can be halted while more lasting solutions are sought.

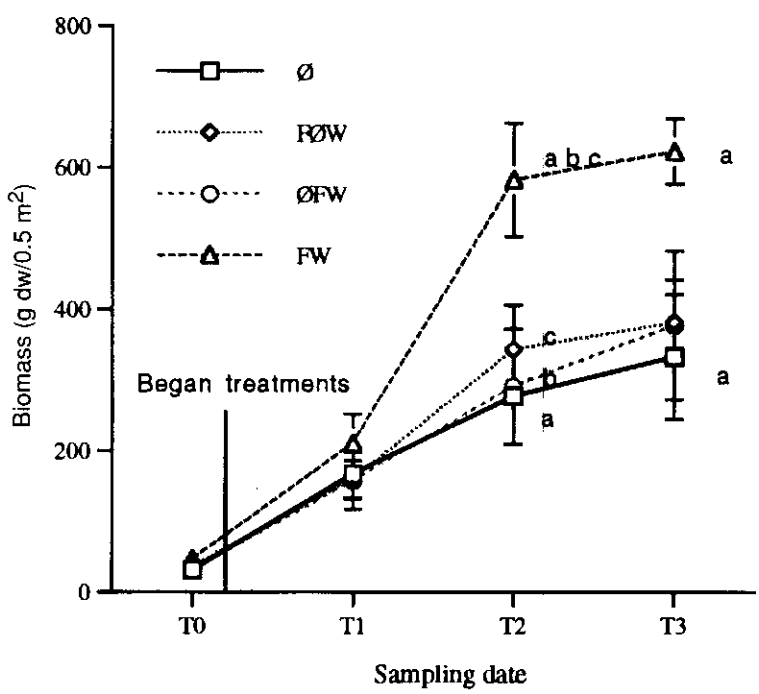

Figure 1. Mean estimated biomass of *Spartina foliosa* within each treatment through time. dw = dry weight; Ø = control, FØW = fertilizer, ØFW = water, FW = fertilizer and water; T0 = March, T1 = May, T2 = July, T3 = September (all 1994); bars show standard error.

Cooperating Organizations

University of California, San Diego
Natural Reserve System

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Causative Factors of the Death of Black Abalone

University of California, Davis
R/F-22-5PD
1993

Ronald P. Hedrick, Caroyln S. Friedman

Beginning in 1985 dramatic reductions have occurred among populations of black abalone (*Haliotis cracherodii*) at Diablo Cove and several Channel islands (Santa Rosa, Santa Cruz, and Anacapa). The cause of the reductions is a progressive atrophy of the foot, or withering syndrome, of affected abalone that eventually leads to their detachment from the substrate and death. The apparent spread of the disease to populations of black abalone on additional Channel islands (San Miguel, Santa Barbara, and San Clemente) led observers to conclude that the mortality might be due to the spread of an infectious agent (Haaker et al., 1992; Vanblaricom et al., 1993). Initial pathological investigations of affected abalone at Diablo Cove and from Santa Rosa Island indicated a heavy parasitism with a renal coccidian (Steinbeck et al., 1991). However, recent studies conducted by us and Armand Kuris at the University of California, Santa Barbara, show a strong correlation between the presence and intensity of the renal coccidian in infected abalone and mortality or condition indexes. Although the renal coccidian may contribute to the mortality, and was a natural focus for initial studies, other factors are most certainly involved. Our pathogen containment facility at the Bodega Marine Laboratory can be used to conduct trials to determine whether the disease is transmissible from infected black abalone to other black abalone.

Objectives

The overall objective of this project was to determine if deaths among populations of black abalone are due to an infectious agent. The objectives for 1992–1993 were to test the transmissibility of the disease by either cohabitation or injections of filtered and unfiltered tissue extracts from infected into healthy black abalone.

Progress Towards Objectives

Two experiments were done to examine the potential transmissiblity of withering syndrome among black abalone. In the first, 100 animals were collected from San Nicolas Island, a site believed to be free of the syndrome at the beginning of the study, and an additional 75 were collected from Santa Rosa Island, where the disease is enzootic. The following treatments were arranged to examine the transmissiblity of the disease by cohabitation of control and affected animals. Two 130-l aquaria were each stocked with 25 abalone from San Nicolas Island and 25 from Santa Rosa Island. One additional aquarium was stocked with 25 abalone from Santa Rosa Island. Two other aquaria each received 25 abalone from San Nicolas Island. The tanks received flow-through full-strength filtered seawater at a constant temperature of 18°C, and the abalone were fed a diet of *Macrocystis pyrifera* previously treated with iodophor for surface disinfection.

After 4 months, a majority of the Santa Rosa Island animals in both the control group and those mixed with the San Nicolas Island abalone had died, with signs consistent with withering syndrome. Only 3 of the animals died in each of the San Nicolas Island control aquaria during this period. Beginning at 5 months many deaths occurred in the San Nicolas Island control groups not exposed to affected abalone. The deaths in the San Nicolas Island control groups corresponded to the parallel observation of the first cases of withering syndrome appearing on San Nicolas Island. At the end of the first year of the study, nearly all abalone in the San Nicolas Island group were dead.

Because of the mortality among control animals, we could not determine transmissibility from this experiment, but we were able to determine that the incubation period for the disease acquired by natural exposures is at least 5–7 months. Presumably the San Nicolas Island animals were already affected but showing no signs of disease when collected for experimentation and later showed evidence of the disease during the experimental period.

A second experiment was initiated in which hemolymph was removed from abalone from Santa Rosa Island showing withering syndrome and used to inject two groups of 30 Ano Nuevo Island (normal) abalone. An additional 30 Ano Nuevo abalone received an equal volume of saline via the same route. The animals were maintained as described in the preceding experiment. The experiment was 17 weeks in duration. The resultant mortality and change in weight of the three groups is shown in Table 1. Deaths and signs of withering syndrome occurred among the two experimental groups but not the control group. Among experimental animals in both groups, even animals that did not die had an appreciable weight loss when compared with controls. By 17 weeks the control animals began to lose weight but at a rate less than that of the experimental animals.

These results, although preliminary, suggest that some agent is being transmitted with the hemolymph that can mimic or reproduce the signs of withering syndrome. The much faster onset of mortality and signs of infection than those observed in San Nicolas

Table 1. Deaths Among Black Abalone Injected with Hemolymph from an Abalone with Withering Syndrome.

Time after Injection (weeks)	No. of Deaths: Experiment 1	Experiment 2	Control	Wt. Dif. (g)
8	6	3	0	7.7
12	6	3	1	7.6
17	6	3	1	3.3

Note: Wt. Dif. = differences in the average weight of the experimental and control aimals. Each of the three groups consisted of 30 animals.

Island abalone in the cohabitation experiment might be due to the direct route by which the animals were infected. A second injection experiment is under way to confirm or refute the hypothesis of an infectious transmissible agent. A second cohabitation experiment is also under way in an effort to obtain data uncomplicated by deaths among control animals.

Cooperating Organizations

California Department of Fish & Game
Channel Islands National Park Service
National Biological Survey
UC Santa Cruz

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Presentation

Kismonhandaka, G., C. Friedman, W. Roberts, and R. Hedrick. Investigations of physiological parameters of black abalone with withering syndrome. Presented at the National Shellfish Association, Portland, Oregon, July 1993.

Education

Education

California Sea Grant's commitment to education is evident in the projects it supports for student at all levels.

The Trainee Program

Research projects supported by California Sea Grant College System generally include at least one graduate student trainee. The students work alongside university scientists and engineers in stimulating research environments, while working on or completing graduate degrees. These young scientists and engineers will be responsible for maintaining the high quality of U.S. marine research in the future.

Isaacs Scholarship

The twelfth John D. Isaacs Memorial Sea Grant Scholarship was awarded in 1992 to Kenia Whitehead, then a graduate of Samuel Gompers High School in San Diego, who focused her research on the effects of ultraviolet light on oceanic phytoplankton. Whitehead is now commencing her Ph.D. studies in chemical oceanography the University of Washington. The 1993 scholarship went to Bryan White of Robert Louis Stephenson High School in Pebble Beach. White built a fiber-optic hydrophone sensor designed to measure animal noises, marine noise, and ocean currents. He is presently a senior at Stanford University. 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. It is selected from other marine-related projects at the annual California State Science Fair.

California Sea Grant State Fellowship Program

This program provides a unique educational opportunity for graduate students who are interested in both marine resources and the policy decisions that affect those resources. The program matches highly motivated and qualified graduate student with "hosts" in the California state government or in state agencies for a nine-month paid fellowship. In1992–93, there were two fellows: Mark Evans from UC Davis, worked with the Pacific Fisheries Legislative Task Force; Melody Paige Tate, also from UC Davis, worked with the Senate Committee on Natural Resources and Wildlife. In1993–94, there were two fellows: Shannon Erickson of UC Santa Cruz, worked with the Joint Committee on Fisheries and Aquaculture; and Linda Martello, also of UC Santa Cruz, worked with California Coastal Commission.

Continuing Projects

Sea Grant Extension Program

The work of the California Sea Grant Extension Program (SGEP), which comprises seven area advisors and two specialists, is organized into four major program areas: Marine Fisheries, Seafood Technology, Coastal Resources, and Aquaculture.

Marine Fisheries

Advisors and specialists of the California Sea Grant Extension Program (SGEP) have divided the Marine Fisheries program into four subprogram areas based on needs and available resources: (1) Fisheries Efficiency and Safety, (2) Fisheries Utilization and Management, (3) Fisheries Enhancement, and (4) Fisheries Education.

Fisheries efficiency project. Improving fishermen's energy efficiency is a major emphasis of this subprogram. Fuel costs are high for most California commercial and commercial-passenger fishing vessels. Increased fuel efficiency would lower harvesting costs and increase profitability. SGEP initiated this project in 1986 and its educational program, linked with that of the California Energy Extension Service (CEES) low-interest loan, resulted in an average fuel savings of 17% for participants. Early in 1992, based largely on its evaluation of program effectiveness, the governor signed legislation (SB1032) authorizing a new $1 million five-year low-interest revolving loan fund. SGEP is continuing its education and evaluation work. The objectives of this long-term project are to increase the awareness of commercial fishermen in adoption of fuel-saving technology and practices, and to assist industry and the state in publicizing and evaluating the energy loan program. Cooperators include the CEES, a fishing industry advisory committee, the Department of Naval Architecture (UC Berkeley), and the Canadian Department of Fisheries and Oceans.

SGEP staff informed vessel owners about fuel saving technologies and the availability of low interest loans through articles in trade journals (*National Fisherman*), newsletters, mailings, and personal contact. Thirty people have applied with three approvals totaling $72,500 (six were denied, and 21 are pending). Requests include engine replacement, hull modifications, efficient propellers and trawl doors, and self-polishing paints. Loans are still available through this CEES $1 million revolving loan fund.

Fisheries safety project. Loss of both lives and fishing vessels at sea has been extraordinarily high for decades. SGEP efforts have focused on the especially difficult safety problems of Vietnamese fishermen and the new U.S. Coast Guard safety programs. The objective of this project is to save lives and vessels.

The new U.S. Coast Guard regulations require onboard safety drills and increased safety equipment. Susan McBride coordinated and served as a water safety instructor for a three-day program in Eureka that certified 353 fishermen in 1994. Leigh Johnson publicized state-funded safety training workshops held throughout southern California. Deborah McArdle brought together UCSD researchers and fishermen to resolve conflicts about the location of a research mooring buoy in the Santa Barbara channel.

Fisheries utilization and management. In recent years, limited entry, individual transferable quotas (ITQ), harvest refugia, and trap certificate schemes have been implemented. Fishery managers and the industry need to be involved in, and informed about, management options, issues, and current research on the effects of these new schemes.

Innovative fisheries management techniques. Chris Dewees, Natural Resource Economist Jim Wilen, and graduate student Keith Casey completed field work on Dewees' NMFS grant on effects of ITQs on the halibut fishery in British Columbia and Alaska. Over 50 processors were interviewed, and vessel owners were surveyed by mail. Preliminary British Columbia data indicate that smaller processing firms specializing in halibut are taking market share away from larger firms. The product mix in Canada has shifted from 90% frozen, before ITQs, to 95% fresh after implementation.

Rick Starr continued his submersible research, and used data from other scientists, to show that rockfishes are more habitat-specific than was expected. This means that rockfish abundance is lower than previously believed because the available habitat for individual species is less prevalent. Starr chaired a session on *in situ* sampling techniques at the Western Groundfish Biologists annual conference. He also participated in annual workshops of rockfish researchers to help coordinate studies by west coast rockfish biologists, and worked at sea with the Alaska Department of Fish and Game, U.S. Geological Survey, and Rutgers University biologists in the evaluation of habitat types to ensure that all west coast rockfish researchers are using a similar habitat classification system. Starr has been assisting Mary Yoklavich of NMFS, Greg Cailliet of Moss Landing Marine Labs (MLML), and Bob Lea of California Department of Fish and Game (CDF&G) in their studies of deepwater rockfishes off Monterey

to compare population structure in fished versus unfished areas. In addition, Starr received a grant from the National Undersea Research Program to study habitat associations of shallow water rockfishes. Early results suggest there is a sharp difference in species use of habitats between water depths of 50–80 m—information that will benefit resource managers and recreational fishers as nearshore rockfishes are the mainstay of this fishery.

Jim Waldvogel continued as a technical advisor to the Klamath Management Zone Coalition. Information he provided helped to establish a salmon sportfishery in the Klamath Management Zone when the rest of the Oregon coast was closed to ocean salmon angling in 1994. Waldvogel also served as council member and co-chair of the Smith River Advisory Council (SRAC), a group influencing the research and fishery management of that system, and wrote their goals and objectives. He chaired and moderated the Smith River Fishery Colloquium, a cooperative conference of the SRAC and the Humboldt Chapter American Fisheries Society. McArdle organized a meeting of academics, representatives of commercial fishing, and seafood processor/retailer industries, to identify Morro Bay groundfish management issues, and develop ideas for applied research studies. Commercial fishers also suggested methods to become involved in research study development and data collection.

Nearshore dive fisheries. With the increased intensity of nearshore dive fisheries, SGEP staff have become more involved. The commercial sea urchin fishery—now the state's most valuable at $25 million per year—is experiencing sharp declines in the mid-1990s.

SGEP staff continue to provide leadership to California Sea Grant's Kelp Bed initiative. McArdle, Starr, and Dewees planned and coordinated a statewide workshop with researchers to discuss priorities and encourage multi-disciplinary research approaches. McArdle edited the workshop summary. Dewees and Charles Igawa (Sea Urchin Processors Association) completed a review of all industry-funded sea urchin enhancement research. The California Fish and Game Director's Sea Urchin Advisory Committee is utilizing this document to guide future research funding policy. Two graduate students supervised by Dewees finished theses on sea urchins. Sea Grant trainee Julie Reynolds (Wilen, co-major professor) did her M.S. thesis on fishery economics and will continue working towards a Ph.D. Laura Rogers-Bennett studied morphology, ecology, and culture of red sea urchins for her doctorate. Graduate student Rogers-Bennett and Dewees completed industry-funded research examining methodologies to mass produce juvenile urchins for enhancement projects. They were able to accelerate settlement to 25 days consistently at high feeding rates. Post-settlement mortality and slow growth were identified as important barriers to successful culture for enhancement. Dewees and McBride (alternate) continued to serve on the CDF&G Director's Sea Urchin Advisory Committee and supplied research-based information to assist the committee with proposed major management policy changes. They also continued to publish the *Sea Urchin, Kelp, Abalone* newsletter.

Increasing recreational use of underutilized fish and underutilized fishing opportunities. The recreational fishing industry throughout California has expressed a strong need to encourage the use of underutilized species, improve the quality of fish landed, and attract more customers. These needs began to be addressed in 1986 with National Marine Fisheries Service Saltonstall-Kennedy funding.

The design of a suitable onboard chilled seawater refrigeration system was tested and demonstrated on a northern California commercial passenger fishing vessel.

Fisheries enhancement Deterioration of freshwater habitat has severely affected salmonid fisheries. The objectives are to improve the knowledge and skills of public groups involved in fisheries enhancement projects, and form coalitions of fishers, agencies, agriculture, and universities to attack problems of critically low-level salmon populations.

Salmonid resources. Dewees and Leon Davies continued to work with the Spring-run Chinook Salmon Workgroup comprised of fishers, landowners, farmers, forest managers, irrigation district representatives, environmental organizations, and resource agencies. Ten workgroup meetings were organized and conducted leading to numerous habitat improvement actions which, to date, have made petitioning for Endangered Species status unnecessary. These improvements helped keep the California ocean salmon fishery open in 1994, generating approximately $10 million in revenue. Group actions also led to a $300,000 allocation through Congressman Fazio's office for Spring-run salmon habitat restoration. Dewees was able to obtain funding from the California Salmon Stamp Committee ($21,371) and the Renewable Resources Extension Act ($9,423) to continue workgroup activities and to train UC extension advisors and specialists in salmon/agriculture issues. Waldvogel edited and distributed 325 copies of the *Proceedings of the 12th Salmonid Restoration Conference*. He also served as technical advisor to the newly established Chetco, Winchuck, and Curry County Watershed Councils. McArdle worked with both the Morro Bay Management Steering Committee and the Morro Bay Regional Water Quality Control Board to incorporate fishing industry concerns into development and environmental issues in the Morro Bay estuary. McBride moderated and coordinated a workshop with the Pacific States Marine Fisheries Commission on Productive Fish Habitat and Sustainable Fisheries. The topics presented and discussed were the Klamath River Estuary, the Eel River Delta, and the Mad River.

Salmonid management studies. The designation of the Smith River watershed as a National Recreational Area in 1991 has increased the need for long-term historic

fishery data to manage salmonid fisheries on the Smith and Winchuck Rivers in northwestern California.

Waldvogel completed the fourteenth year of a 20-year escapement study for chinook salmon on Mill Creek (Smith River). Fishery data from this ongoing study was a major factor in rejecting a proposed CALTRANS highway 101 bypass in the Mill Creek watershed—a savings to taxpayers of over $30 million. Waldvogel completed adult steelhead scale sampling on the Winchuck River during which the continuing appearance of hatchery strays persisted throughout the ten-year study period. He also completed the fifth consecutive year of analyzing adult chinook returns to Rowdy Creek Hatchery (Smith River) for age and growth, and conducted a survey with more than 200 in-river salmon and steelhead sport fishermen concerning fishery issues, management techniques, and user conflicts.

Marine fisheries education. The overall objective of this subprogram is to supply research-based information to Californians to increase their understanding of fisheries and fisheries issues.

All SGEP staff respond to hundreds of requests for fisheries educational materials and information. McArdle provided training on commercial fisheries and harvest refugia to 20 Pacific Coast teachers participating in the Pathfinder Program funded by the U.S. Navy, NOAA and the Department of the Interior. The training program was organized by USC Sea Grant. Waldvogel participated in the International White Shark Symposium, organized the education/media sections of the symposium, and wrote and edited the educational section of the proceedings. McBride collaborated with the Salmonid Restoration Federation in their Watershed and Fishery Resource Conservation Education Network Program to produce a resource reference guide of educators, agencies, private organizations, and individuals involved in fishery restoration. McBride developed the database for the mailing list and assisted with survey development for network participants. She also participated in fisheries outreach projects for presentation to Native Americans and a Women in Natural Resources group. Dewees co-taught a graduate course in fisheries management at UC Davis with 12 students from ecology, economics, and environmental policy. He also organized a marine fisheries session at the Pacific Fisheries Biologist meeting at Clear Lake. The publication, *California's Living Marine Resources and Their Utilization*—the result of a major effort in 1992 by SGEP—continues to be widely used by agencies, legislators, industry groups, environmental organizations, educators, and others. Approximately 950 copies have been sold so far. A good example of the benefits of cooperative regional Sea Grant activity is the timely distribution of NOAA sea surface temperature charts to 55 fishermen in Eureka to help them locate fish efficiently. In addition the new albacore handling publication by Price (California) and Melvin (Washington) was widely used by fishermen to land high-quality albacore.

Seafood Technology

Extension Seafood Specialist Price and Program Representative Pamela Tom conducted a two-day "Seafood Handling and Quality Evaluation" workshop for processors, retailers, and commercial laboratories. Price also gave six invited presentations on seafood quality topics at workshops, short courses, and training meetings. Following a study that indicated that the cooling rates of fish fillets in cardboard, plastic, and metal containers are directly related to container thickness, Price presented a paper on this subject at an international professional society meeting and chaired a seafood quality session. Price and Tom authored or co-authored eight publications and one-sheet flyers on seafood. Formal evaluations of these training meetings indicated that the information provided was valuable and worthwhile. Price and Tom completed *A Guide to California Seafood* for the California Seafood Council, a major publication containing over 40 color photographs of fish and shellfish species, product description and form, natural history, production, size and grade, quality factors, packaging, labeling, and nutritional value.

Seafood quality improvement. A National Sea Grant priority is to improve the quality of seafood products for consumers. This project aims to ensure seafood quality and safety by encouraging the adoption of improved processing and handling practices, and encouraging industry participation in Cooperative Extension and Sea Grant educational programs.

Seafood safety. Price and Tom conducted three HACCP workshops and gave 32 presentations at workshops, short courses, and training meetings. Formal evaluations of training meetings indicated that the information provided was valuable and worthwhile. Price served on the steering committee for the Seafood HACCP Alliance, comprising federal, state, and local health regulatory agencies, the seafood industry, and Sea Grant. The alliance seeks to ease implementation of the U.S. Food and Drug Administration's proposed mandatory HACCP regulations for seafood processors and importers. Price is chair of the alliance committee to develop a compendium of approved processes and controls, and co-chairs a committee to develop educational materials for allied industries and the public. Marine Advisor McArdle reviewed the Field Sampling Work Plan Related to the Health Advisory Adjacent to the Guadalupe Oil Field for the Santa Barbara County Department of Environmental Health. The study is intended to determine if surf sportfish exposed to the Guadalupe oil spills are safe to eat. The information will be used by the California Department of Health to determine the necessity for a sportfish health advisory notice for Santa Barbara County.

Coastal Resources

California's coastal bays and estuaries continue to experience incursion because of expanding

commercial and residential development, resulting in the loss or degradation of 90% of the state's wetlands. Education and research are essential for decisionmakers and citizens so that wise policies can be adopted on developing, managing, and conserving coastal and marine resources. Interdisciplinary approaches are increasingly needed to meet complex challenges to coastal and marine ecosystems.

Coordination of MBNMS management plans with central California coastal resource management. The objective is to improve coordination of federal, state, and local agency coastal resource management with the national marine sanctuary. Primary cooperators include the Association of Monterey Bay Area Governments (AMBAG), California Coastal Commission, Elkhorn Slough National Estuarine Research Reserve (ESNERR), MBNMS, and major research, educational, and nongovernmental conservation organizations in the central California region.

Marine Advisor Starr is serving on three advisory committees and helping coordinate a water quality protection program sponsored by NOAA's Office of Resource Conservation and Assessment. As a member of the Project Development Team for the water quality protection plan, Starr participated in strategic planning meetings and co-facilitated a large workshop identifying and suggesting solutions to water quality problems. As an education committee member, he recommended ways to increase public awareness of the planning process. Starr wrote two articles for the quarterly newsletter of AMBAG, and two newspaper articles on the water quality planning process for the agricultural sections of the Salinas Californian and the Monterey Herald. He served on the Conservation Working Group, the Research Activities Panel, and the Sanctuary Education Panel for the MBNMS. He co-chaired the organizing committee for the Monterey Bay Research Symposium, which attracted over 250 people. Starr cooperated with Yoklavich (NMFS) to survey fisheries scientists and resource managers on research priorities for MBNMS, and results were incorporated in the sanctuary's research plan. He also chairs the ESNERR Advisory Committee.

Marine protected areas. These areas are being used worldwide to conserve and manage marine resources on an ecosystem level. Approximately 116 marine protected areas exist in California under various designations and levels of protection. Information about this wide array of marine ecosystems and habitat types is scattered and inconsistent. Objectives of this project are to increase scientists', resource managers', and users' access and ability to utilize data on the state's marine protected areas. Cooperators include University of California at Santa Barbara Geography Department (UCSBGD) and CDF&G.

McArdle participated in two international conferences on ecosystem conservation with marine protected areas, and was named an information contact on the International Marine Protected Areas Network. In addition, she cooperated with UCSBGD and CDF&G to develop and submit a research proposal for the creation of a publication, and Geographical Information System (GIS) database on locations, regulations, goals, and resources of California's marine protected areas. These materials will provide comprehensive information for developing habitat and living marine resource management programs.

San Diego Bay environmental management. Some of San Diego Bay's resources are threatened by water and sediment contamination. The San Diego Interagency Water Quality Panel represents 33 federal, state, and local government, military, industrial, commercial, recreational, environmental, and academic organizations, and is mandated to coordinate environmental monitoring, develop a comprehensive management plan, and coordinate public education regarding San Diego Bay water and sediment quality. This project seeks to evaluate panel accomplishments, and improve the ability of its members in developing sound environmental research and management programs for San Diego Bay.

Johnson participated in deliberations at four San Diego Interagency Water Quality Panel meetings. She served on the Management Committee and helped to develop comprehensive environmental goals and objectives for San Diego Bay. Her analysis on the effectiveness of the panel's Fiscal Management Committee during 1988–92 was published in the 1992 annual report. The panel is acting on survey recommendations to increase involvement of policy makers in coordinated monitoring and comprehensive management.

Multiple use of coastal resources. GIS can be used to interpret multiple disparate data sources needed by coastal resource managers for solving coastal resource issues. However, the design and development of GIS is a complicated task, requiring both technical expertise and interagency coordination. The objective is to improve access of agencies and organizations to such technical expertise. Cooperators include AMBAG, California Coastal Commission, ESNERR, and MBNMS.

Starr provided technical advice and information about the use of GIS to help identify and solve central California coastal resource management problems. Starr chaired an AMBAG Technical Advisory Committee to oversee a project in which GIS will be used to solve urban and rural water quality problems. Starr also helped a graduate student develop a GIS for Elkhorn Slough. Starr helped researchers from NMFS and Moss Landing Marine Laboratory (MLML) set up a GIS to analyze multiple data sets related to oceanographic fisheries research. He cooperated with Yoklavich of NMFS who is using sidescan sonar, geological data, and fish distribution data to identify natural refugia for rockfish. Starr also helped set up a GIS for researchers from MLML, ESNERR, and the California Coastal Commission. He cooperated with Rikk Kvitek from MLML in a GIS that would incorporate field vegetation analysis

with NASA imagery to map changes in watershed (particularly wetlands).

Reducing boating impacts on coastal water quality. NOAA, USEPA, state, and local agencies are developing programs to reduce pollution of coastal waters from nonpoint sources (NPS), including marinas and recreational boating. California's boating businesses, boaters, regulatory agencies, and environmental groups need assistance in selecting and implementing NPS management measures. The project objective is to empower boating, environmental, and government interests to work together in reducing boating impacts on coastal water and sediment quality. The San Diego area is the initial focus with partial USEPA funding. State and national cooperators will be consulted and provided with findings. These include USEPA, California Coastal Commission, State Water Resources Control Board (SWRCB), Regional Water Quality Control Board, California Technical Advisory Committee (TAC) for boating-related NPS pollution, and boater, boating industry, and environmental representatives, Oregon Sea Grant Program, and the National Sea Grant Water Quality Network.

Johnson and Program Representative Erika McCoy interviewed 128 representatives of government, boating, and environmental interests on their knowledge, communications, concerns, and recommendations on boating and NPS. Johnson and McCoy conducted a forum in which 35 participants recommended priorities for voluntary and regulatory programs. Evaluations found that participants' knowledge of NPS, communications, and the concerns of others had increased since they were interviewed. Participants indicated they would use project information in managing NPS and that boatyards would have preferred this type of approach when they were regulated by the stormwater program. A 70-reference boating NPS library was assembled for marinas, boaters, underwater hull cleaners, and boatyards. Outlines were reviewed favorably by 44 local, state, and federal participants, members of the California TAC for boating NPS, and SGEP staff in California and eight other states. Reviewers were invited to advise on developing final best management practices and educational presentations. Johnson provided interview and forum findings to the California TAC, co-chaired its educational subcommittee, was invited to chair a committee to recommend a statewide boating NPS education program, and provided master copies of educational materials for statewide dissemination. She presented five invited papers on public policy and consensus education for coastal NPS management to the Environmental Management and Technology Conference, The Coastal Society Conference, USEPA Region IX's Challenge of Watershed Protection Conference, the National Sea Grant Water Quality Workshop and the Eleventh U.S. Coast Guard District's Environmental Summit. In addition, Johnson served on the committee to draft a National Sea Grant Strategic Outreach Initiative on Coastal Ecosystem Health which is targeted for presentation to Sea Grant Directors and MAS leaders in 1994–95.

Eureka harbor waterfront revitalization. Economic restructuring in northern California has led to declines in resource-based industries, as well as to growth of service-related and high technology industries, innovations and centralization of water-borne transportation, changing demographic characteristics, and changing amounts of leisure time and activities. Coastal businesses such as commercial fishing and aquaculture have lost much vital infrastructure in Humboldt Bay. Waterfront revitalization offers opportunities to stimulate economic development in coastal northern California communities. The objective is to increase fishing and aquaculture industry participation in planning and implementation of the Eureka Harbor Commission Waterfront Revitalization Program. Cooperators include the Eureka Harbor Commission Waterfront Revitalization Committee, U.S. Coast Guard, and numerous marine and waterfront businesses and interest groups.

Marine Advisor McBride trained 34 community members using slide presentations and waterfront walks to promote awareness of the Eureka Waterfront Revitalization Program and similar activities in other communities. Data gathered locally and from other areas was used to establish criteria for commercial fishing industry storage and repair areas and dockage. This was collected via individual contacts, meetings with community groups, and a survey of volunteers from the Eureka Main Street program. McBride also made invited presentations to the Citizens for Port Development and Eureka Kiwanians Club on a number of topics including waterfront revitalization progress, funding for large docks, and descriptions of other small coastal town waterfronts in the Pacific northwest.

California coastal earthquake hazards management. Earthquakes and tsunamis threaten lives, coastal structures, and property, so citizens and decisionmakers need to understand the threats posed by these hazards and ways to reduce risk through better planning and development.

McBride collaborated with Oregon Sea Grant Specialist Jim Good, Washington Sea Grant Specialist Bob Goodwin, and a number of state universities and agencies to plan a September 1995 regional conference, "Cascadia Earthquakes and Tsunamis: Implications for Coastal Management" to investigate integration of earthquake-related considerations in land-use planning and development.

North central California coastal watershed management. Coastal resources is this region are affected by urban and agricultural populations. The objective of this project is to increase decisionmakers' and coastal watershed users' access to research-based information. Cooperators include the Tomales Bay Advisory Committee (TBAC) and Shellfish Technical Advisory Committee, California Department of Health Services (CDHS), and numerous resource user groups.

Marine Advisor Paul Olin met with the TBAC to determine water quality research needs and participated in

TBAC meetings to discuss effects of ranching, dairies, and other agricultural activities on water quality and sedimentation in the bay, where erosion, in the last decade, has decreased the tidal prism by 25%. Olin also discussed alternate land uses and opportunities for diversification, including llamas, organic farming, and dairies. He cooperated with CDHS in monitoring Tomales Bay phytoplankton and water quality and provided them with a microscope for an algae identification workshop. He gave information on watershed protection and enhancement to resource managers and grassroots organizations at the Redwood Coast Watershed Conference.

Coastal resources education. A continuing objective of Waldvogel and Olin is to increase the access of northern California marine and coastal resource users, decisionmakers, educators, and youth to research-based information on marine and coastal resources and issues.

Waldvogel edited and wrote the Educational Section of the International White Shark Symposium Proceedings. Marine Advisor Olin contributed to a *Skin Diver* article on the effects of feeding wild fish in marine waters. He provided information on fish nutrition, dietary requirements, protein-to-energy ratios, and conditions resulting from nutritional deficiencies. Olin and Cooperative Extension Youth Advisor Dan Desmond identified teacher inservice needs and planned training on aquaculture, and other agri-environmental curricula.

Fort Bragg High School Research Center. The town has experienced social and economic stress from commercial salmon season closures, reduced sea urchin landings, timber industry decline, and construction industry job losses. The high school developed a research center to address community problems through research and upgraded curriculum. Objectives are to increase this center's ability to develop research projects, educate students, and address community problems.

McBride cooperated with the Fort Bragg High School Advisory Committee in a presentation to California Department of Education staff for the high school's successful application for second phase funding from the Specialized Secondary Program. McBride also trained three high school teachers on seawater systems and sea urchin and abalone culture methods.

Central California coastal resources education. As a result of the establishment of a National Marine Sanctuary in 1992, the project aims to increase information access by area residents to the sanctuary's resources, values, and goals. Cooperators include Pandion Enterprises Wildlife Photographers, MBNMS, Center for Marine Conservation, and other conservation and education organizations.

Starr collaborated with Pandion Enterprises Wildlife Photographers and the Corporation for Broadcasting (PBS) on a film about sea otters. This aired on PBS in March, 1994 to an audience of 10 million. Part of the film is shown daily at the Monterey Bay Aquarium, reaching 5,000 people per month. Starr also designed, developed, and distributed 55,000 educational brochures related to the MBNMS. He cooperated with sanctuary staff, businesses, and non-governmental groups to provide brochures to 30 marine user groups on conducting recreational activities without harming sanctuary resources. The brochures are displayed at 25 interpretive centers with over 500,000 visitors per year.

Southern California marine and coastal resources education. A continuing objective of Johnson is to increase access of southern California marine and coastal resource users, decisionmakers, educators, and youth to research-based information on marine and coastal resources and issues.

Johnson provided ocean and coastal resources information to 920 marine educators, industry members, scientists, decisionmakers and the public through the quarterly newsletter *Tidelines*, and cooperated with California Sea Grant Information Specialist Gretchen Frederick to present seafood consumer, marine education, and general Sea Grant information to 300 people at San Diego's Day at the Docks festival. Johnson demonstrated sea urchin research techniques to 107 middle school youth, and distributed Isaacs college scholarship applications, and ocean career publications. This information, together with details of sportfishing quality control research, was also given to 600 high school students and teachers at the Science and Technology Careers Fair organized by Congressman Randy Cunningham.

Aquaculture

The objectives of the aquaculture program are to facilitate the development of new technologies and extend new research-based information to abalone, oyster, and salmonid aquaculturists using a variety of methods.

Aquaculture public service. Coastal residents interested in aquaculture need access to sources of information for making investment decisions, and this public service is a continuing activity of the SGEP. McBride gave presentations on California coastal aquaculture to local public television and radio stations, university students, and at a regional Alternative Aquaculture Conference sponsored by Cooperative Extension. She hosted a nationwide teleconference on Aquaculture Quality Assurance in which fish and shellfish aquaculturists heard producers from a wide variety of U.S. aquaculture products and quality assurance experts. McBride also developed an exhibit of live oysters, clams, and mussels at all life history stages in a recirculating water system with brightly colored laminated interpretive signs. The display was used at two events this year. Olin assisted with a State Health Department training workshop and provided a projecting microscope to use during the phytoplankton identification session.

Water quality and oyster farming. Following rainfall, pollution is washed down each watershed into shellfish-growing waters. Unfortunately, rain occurs mostly during winter months—the height of

the harvest season. Dairy waste affects shellfish beds in Humboldt and Tomales Bays.

Olin worked with Tomales Bay shellfish growers, and state and federal health departments to develop depuration and holding systems for oysters. He also assisted in a study to monitor water quality related to oyster lease closures in Tomales Bay. This study provided valuable information to determine the effects of livestock and septic systems on water quality.

Economic diversification in Humboldt County. Aquaculture has possibilities for diversified uses of coastal resources to benefit a variety of users and protect the environment. In the coastal zone land owners are interested in utilizing their freshwater resource for culture of rainbow trout.

McBride worked with 15 rural landowners to establish the Northern California Aquaculture Cooperative. She organized three training days for cooperative members to collect broodfish from ponds, index the broodfish for reproductive status, and finally to strip the fish, and rear the eggs and young trout. These efforts resulted low-intensity rearing of rainbow trout for two pay lakes operations.

International Symposia on Abalone Biology, Fisheries, and Culture. Over 200 participants from sixteen abalone producing countries attended this conference in Hobart, Tasmania. Research topics and workshops in the scientific program included international abalone trade and market patterns, genetic considerations for hatchery and wild abalone populations, biotechnology, development of artificial diets, methods to assess stock abundance and juvenile recruits, methods of fisheries enforcement, and cooperative management of fisheries.

McBride presented a paper on the genetic composition of five hatchery and four wild red abalone populations. Estimates of inbreeding and genetic drift within these populations suggest variable success of mass spawned abalone. McBride also served on the organizing committee as the North American delegate for this conference and is helping to plan the 1997 symposium in Monterey.

Abalone aquaculture education/technology transfer. There is continued high interest in the feasibility of abalone aquaculture in northern California and a need to bring researchers together with industry to solve production problems.

During 1993–94, McBride organized an educational workshop which included presentations from two commercial abalone farms, Olin, and a CDF&G biologist and shellfish pathologist. There was also a panel on "Women in Aquaculture" and another which addressed challenges facing the abalone aquaculture industry. A cross-section of perspectives presented the varied experiences of California abalone farms and introduced the regulatory requirements for starting a marine aquaculture business. Fisheries Specialist Dewees brought researchers and abalone aquaculturists together to discuss methods for controlling infestations of abalone under culture conditions. This led directly to a rapid response project, further industry discussions, and a full Sea Grant proposal to examine potential solutions.

Aquaculture information flyers. Non-profit habitat restoration organizations are very active in northern California counties. These groups continually request information from Sea Grant Advisors.

Advisor Waldvogel continued development of computerized information flyers related to volunteer rearing of salmonids in net pen culture, enhancement of razor clams, and sturgeon aquaculture. He is cooperating with UC Cooperative Extension Aquaculture Specialist Fred Conte who received funding for this project from the Western Region Aquaculture Consortium.

California, which is now home to 31 million people, 80 percent of whom live in coastal areas, stretches for more than 1,000 miles along the Pacific.

Not surprisingly, the state has developed a strong academic tradition in marine science. From modest turn-of-the century beginnings in San Diego under a University of California professor from Berkeley, marine research in the University of California has developed into the world's largest and most diverse academic program in ocean science and technology.

Today, on all eight general campuses of the University, marine studies are integrated into many departments. And on five of these campuses—Berkeley, Davis, Santa Barbara, Santa Cruz, and San Diego (i.e., the Scripps Institution of Oceanography)—there are also units devoted solely to marine studies.

In addition, there are strong marine science curricula at a number of private universities, including Stanford University, and at several of the California State University (CSU) campuses, including Humboldt State University, San Diego State University, and Moss Landing Marine Laboratories (sponsored by a consortium of CSU campuses).

In California, Sea Grant began in 1968 with an award to Scripps Institution of Oceanography. By the following year, the National Sea Grant Program was supporting separate projects as well at San Diego State University and UC Santa Barbara. Ultimately, in order to achieve greater coordination and reduce administrative expenses, programs at the various University of California and California State University systems consolidated into the University of California Sea Grant Program. In 1973, this program was designated a Sea Grant College "for sustained excellence in research, education, and public service dedicated to wise use of America's marine resources."

Today, the California Sea Grant College System is the largest in the national network, with a reputation for supporting strong, cutting-edge research in marine science and technology. In the period from FY 1992–94, the program supported 83 major research projects, plus rapid response projects as well, in the general areas of Coastal Ocean Research, Aquaculture, Fisheries, New Marine Products, Ocean Engineering and Instrumentation, and Marine Affairs. The projects are selected on the basis of competitive proposals.

In addition to research, the California Sea Grant College System has an active extension component and a range of educational programs, chief among which is graduate training through its trainee program.

Communications Objectives

The Communications Office is integral to California Sea Grant's mission of sustainable development of our nation's coastal and marine resources. Like its counterparts around the nation, California Sea Grant's Communications Office works to make the program widely known and understood, to disseminate information about the program's accomplishments, to increase understanding of marine-related issues, and to help the program communicate with its diverse audiences.

Communications goals can be formally stated as follows:

- To inform a wide spectrum of constituencies about the mission and activities of the state, regional, and national Sea Grant programs;
- To inform a wide spectrum of constituencies about the scientific findings and other accomplishments arising from Sea Grant-sponsored projects;
- To educate a wide spectrum of constituencies about state, national, and international marine resource issues;
- To support the communications needs of program management, thereby contributing to Sea Grant's visibility and effectiveness.

Operations

Mechanisms for information gathering. Perhaps the single most valuable formal mechanism that the Communications Office has for gathering information is the Annual Progress Report, requested of each project leader every October 1. The Annual Progress Report has three components: (1) a questionnaire, (2) a technical narrative report, and (3) a trainee report.

The questionnaire asks project leaders about results-to-date in light of project goals and objectives, practical applications of their work, press contacts, publications to date, cooperating organizations, international contacts, and so on. This document is used in a variety of ways. Here are just three:

The questionnaire alerts the communications manager to accomplishments that should be followed up and reported to the media or to federal or state agencies;

It helps us track what media coverage a project has received, either as a result of our efforts, or those of the project leader and his or her university public affairs office;

It provides lists of published articles, books, and conference presentations that result from Sea Grant-funded projects. This ensures that our publications inventory and archives are kept as complete and up-to-date as possible.

Another important component of

the Annual Progress Report is the technical narrative. Reports of completed projects are published in a Biennial Report of Completed Projects. This publication not only describes overall project accomplishments, but also provides a forum in which project leaders can discuss difficulties encountered, project modifications, public benefits, and so on. The *Biennial Report* contains lists of cooperating organizations and also a complete list of publications resulting from the project.

Management of information. In order to encourage project leaders to report their publications to us (and to appropriately acknowledge Sea Grant support), the Communications Office pays for 100 journal article reprints, 50 of which we give to the author and 50 of which we keep for mandatory and other distributions.

In any given year, the research projects funded by California Sea Grant result in the publication of as many as 50 to 80 specialized articles in refereed journals, plus other publications such as theses and dissertations, technical reports, and conference papers. The fact that California Sea Grant scientists have the highest publication record in the network has ramifications throughout the area of information management and dissemination and necessitates our having a full-time information specialist.

The Information Specialist has responsibility for maintaining records on publications, for filing and distributing publications, and for filling requests. Specifically the Information Specialist:

(a) Maintains a cumulative, computerized inventory of Sea Grant publications dating back to 1968. There are at present 2,554 entries in this system.

(b) Maintains a physical archive of these publications, which also includes those produced by Extension specialists and advisors;

(c) Maintains a sophisticated computerized database of 5,000 names and addresses organized into 120 specialized groups. This database allows California Sea Grant to achieve highly targeted mailings—for example, to state legislators or to members of the Pacific Fishery Management Council or to high school teachers in Santa Barbara County. Much of the information specialist's efforts go into list maintenance;

(d) Maintains a "library" of journals, newsletters, and brochures that the program receives from other sources;

(e) Tallies the number of publications received and distributed each month, and enters this information into the computer. A spreadsheet produced from these data details monthly distribution and requests counts by type of publication. At the end of each quarter, a report is generated consisting of the spreadsheet with monthly and year-to-date totals, an initial distribution analysis for publications received during the quarter, year-to-date totals of initial distributions, and a page of distribution notes commenting on noteworthy requests and distributions during the period. A listing showing how many times each publication was requested and/or distributed each month during the quarter is also included.

The Information Specialist also has a critical role in information dissemination: (a) She handles distributions of reprints, announcements, press releases, and California Sea Grant series publications, and fulfills all mandatory distribution requirements, including those for Extension materials; (b) She answers all phone, mail, and "walk-in" requests for general and specific information and fills all orders; (c) Twice yearly she compiles and distributes Publications Lists containing newly received publications resulting from our programs. Each mailing goes to approximately 2,100 persons.

In FY 1992–94, the Information Specialist distributed reprints of 125 journal articles and 16 papers from published conference proceedings. In addition, she distributed publications in the California Sea Grant series and miscellaneous publications in a number of categories, for a total of 205 different items, or 18,990 pieces. Publication announcements, press releases, and awards announcements brought the number of pieces distributed to 91,192.

The Information Specialist not only handles initial distribution of publications, but also maintains a library of reprints and books from which to fill both specific and general requests for information. In 1992–94, there were 2,491 individual requests for information or publications, bringing the total number of pieces distributed to 107,829.

Information Dissemination

Once information on marine issues or program accomplishments has been gathered, the communications manager, acting in consultation with the program director, decides on appropriate methods for disseminating it. There are a number of communications vehicles that are used routinely and are thus identified in this document as "core" publications. These are the *Program Directory*, *Summary Report*, and the *Biennial Report of Completed Projects*. In addition, conference summaries and proceedings, technical and working papers, specialized publications, a bimonthly newsletter (*Sea Grant In Brief*), press releases, and announcements are regular products of the Communications Office. These will be described more fully in the Progress Report and Proposed Activities sections of this proposal, which follow.

Cover design is usually done by freelancers or by the design department at the University of California, San Diego, under the direction of the communications manager. Word processing, typesetting, and page preparation and layout are done internally using Microsoft Word, Pagemaker, FileMaker Pro, and Freehand. Printing is either done by the University or by outside vendors on the basis of solicited bids in accordance with University policy.

Publications

In 1992–94, we produced the following publications:

California Sea Grant Program Directory, 1993–94. 1993. 24 pages. The *Program Directory* is a core publication, distributed to all 5,000

persons on our mailing list and thus to audiences as diverse as marine industry members, legislators, scientists, and educators. This publication serves the program's need for a brief overview of current activities written in lay language. It provides readers with an appreciation of the potential application of strong, basic science to pressing marine issues.

Sea Grant in California: Promoting Coastal Ocean Science and Education. (Program Summary Report.) 1993 (40 pages). The Program Summary is a core publication, distributed to all 5,000 persons on our mailing list and thus to audiences as diverse as marine industry members, legislators, scientists, and educators. It serves the program's need for a general, easily comprehensible introduction to the program, directed to the broadest possible audience.

The report included a bound, return-postage-paid Reader's Survey Card, designed to allow us to evaluate such factors as: percentage of readers representing different identified audiences; issues of greatest concern to them; how much of the report they read; how many people share each publication; the level, length, and readability of the feature articles; the usefulness of information presented; and the degree to which the report helped readers to better understand California Sea Grant. Three open-ended questions were included to ensure two-way dialogue with readers. Among the survey results were the following: 75% of those responding read all or most of the publication; in 66% of cases the publication was read by one or two people, and in 21% by more than 5 people. In addition, 97% rated it very or moderately interesting; 91% rated it informative (as opposed to too simple or too technical); 92% found the information very or moderately useful; 89% found it helpful in understanding Sea Grant.

This publication received a Special Merit Award from the Public Relations Club of San Diego in 1994.

California Sea Grant Biennial Report of Completed Projects, 1990–92. 1994 (257 pages). This publication serves the need for accountability for public funds expended by the program. It documents both the successes and failures of different avenues of scientific exploration and includes a record of refereed publications produced as a result of this work, thereby contributing to scientific advancement. It is widely distributed to the marine scientific community, to university and specialized libraries, and to resource managers.

Ensuring Food Safety...The HACCP Way: An Introduction to HACCP & A Resource Guide for Retail Deli Managers, by Robert J. Price and Pamela D. Tom of the California Sea Grant Extension Program and Kenneth E. Stevenson of the National Food Processors Association. 1993 (40 pages). The Hazard Analysis and Critical Control Point (HACCP) system for ensuring food safety has recently been mandated by the U.S. Food and Drug Administration. This publication provides managers and workers in food processing with extensive education about the philosophy behind this system, how it can be implemented, and what educational resources are available. As indicated by the title, the publication is intended primarily for retail delicatessen managers, with the preparation of seafood salad used as the primary example. The text presents very basic HACCP concepts, however, and the resource guide in the appendix is extensive; hence, the publication represents a good general introduction to the topic for anyone in the food processing industry.

The publication was prepared in collaboration with an advisory committee that included representatives of industry (e.g., Safeway) and management agencies (e.g., The California Department of Health Sciences), and was extensively reviewed, rewritten, and revised prior to publication. It was jointly published by the California Sea Grant College System and the U.S. Department of Agriculture. The extramural support enabled our program to distribute 50 copies *gratis* to other Sea Grant programs around the country (for a total of 1,500 copies; with additional copies available for $1 each). In addition, we listed the publisher as the National Sea Grant College Program, rather than California Sea Grant, an action that we believe has contributed to its broad national distribution.

Publication announcements were broadly disseminated, review copies were sent to major national seafood trade magazines, and ads were run in the newsletters of the California Seafood Council and the California Seafood and Fisheries Institute. Orders from around the country were so strong that a second printing was done in fall 1993, with supplementary funding from the International Dairy, Deli & Bakery Association, which ordered 3,000 copies. Other companies ordering multiple copies of the publication to date include: FESCO (Food Equipment Specialists Co.) (150); Hy-Vee Food Stores (150); Procter & Gamble (110 copies); Fairway Foods, Inc. (60); Alto-Shaam (food preparation and equipment) (50); Department of Environmental Conservation, Alaska (50); Johnson's Wax (30); Elmendorf Air Force Base, Alaska (25); California Department of Public Health (24); Hawaii Department of Health (19); Frito Lay, Inc. (10); Napa State Hospital, California (10); and University of Nebraska Food Processing Center (10).

A Reader's Survey Card designed to evaluate the usefulness of the publication and the appropriateness of the presentation was distributed to those who order the publication, as well as at Sea Grant-sponsored HACCP workshops around the nation. Informal feedback at these workshops suggested the need for translation of the publication into Spanish (see below).

Directory of Academic Marine Programs in California: A Guide to Programs in the Marine Sciences at California Colleges and Universities, Third edition. 1994 (86 pages). We receive frequent requests from parents, students, and counselors throughout the nation for information about the degree programs in

marine science (as opposed to simple course offerings) available at 25 institutions of higher learning in the state. This publication describes programs appropriate for those interested in professional and nonprofessional careers and thus serves to introduce students, parents, and guidance counselors both to the diversity of possible marine-related careers, and to the institutions best suited to prepare students for these careers. This third edition was completely updated on the basis on questionnaires we distributed to community and four-year colleges and universities statewide. In addition to descriptions of the degree programs, research facilities, and courses offered are listed for each institution along with contact persons.

Taxonomy of Economic Seaweeds: With Reference to Some Pacific Species (Volume IV), Isabella A. Abbott, University of Hawaii, editor. 1994 (200 pages). The identity of and relationships between major groups of Pacific seaweeds remains largely unknown, a fact that inhibits commercial exploitation of this important resource. This series of publications utilizes the expertise of a small (and diminishing) population of experts around the Pacific Rim to elucidate the taxonomy of economically important Pacific seaweeds. It contributes to our understanding of marine biodiversity and enhances the potential commercial utilization of algal resources.

Taxonomy is the science of discovering, describing, and classifying species or groups of species. Until recent emphasis on the importance of documenting the earth's biodiversity, taxonomy had largely fallen out of favor in this century. Few new taxonomists were being trained, and existing specialists had difficulty finding academic positions. The population of eminent people in the field was rapidly aging. In 1986, California Sea Grant first began to bring together a small but renowned group of algologists from Pacific Rim nations such as China, Japan, Chile, Korea, the Philippines, and the United States in a series of biennial workshops designed to work out the identity and relationships of Pacific algae with potential commercial importance. At these meetings, some of the world's leading marine algal systematists work together to identify, describe, and classify subtropical and tropical red and brown algae groups, and particularly those that yield useful biopolymers, including agar, carrageenan, and alginate. The workshops, which are organized by Dr. Isabella Abbott of the University of Hawaii, have resulted in this series of authoritative, highly illustrated publications.

The first volume in this series received the Blue Pencil Award from the National Association of Government Communicators, and the series has received excellent reviews in the *Plant Science Bulletin, NAGA*, and *The ICLARM Quarterly.* The fourth workshop, whose results are documented in this volume, was hosted by Hokkaido University in Sapporo, Japan in July 1991. The series of publications has a wide international audience; multiple copies are frequently ordered from Japan, for example. Publications in this series are produced with special supplementary funding from the California State Environmental License Fund.

California Sea Grant Program Directory, 1992–95. 1994 (32 pages). This core publication was expanded to encompass two years' projects, and was published in this format for the first time.

Research Needs on Kelp Bed Resources: An Interdisciplinary Approach, Deborah A. McArdle, editor. 1994 (11 pages). This summary of a program development workshop sponsored by the California Sea Grant College System and attended by biological and physical scientists from around the state calls for an increase in interdisciplinary interactions and identifies research gaps related to recruitment, population dynamics, and community interactions in kelp beds as well as to social, economic, cultural, and management considerations.

We will be publishing *Aseguremos La Confiabilidad de Los Alimentos...A La Manera Del HACCP.* This is in response to requests for a Spanish version of *Ensuring Food Safety...The HACCP Way.* We propose to have the English version translated by a team comprising a food safety inspector and a Spanish-language translator for Cooperative Extension. The translation will then sent for review to five Spanish-speaking industry representatives (in Mexico and the United States). The publication is planned for marketing to Spanish-language food science editors.

We also plan to publish the fifth volume in the series, *Taxonomy of Economic Seaweeds: With Reference to Some Pacific Species.* This is the results of a workshop hosted by the Universityof Hawaii, Honolulu in July 1993.

In addition, we plan to purchase 100 copies of the *Seafood Product Guide* by Robert Price, California Sea Grant's Seafood Technology Specialist. This highly technical publication was published by the California Seafood Council for seafood wholesalers and retailers outside the state (including overseas) who might want to order California seafood, but require information about product forms and quality factors. The publication includes a color photograph of each of 40 species. The text includes product descriptions plus information on packaging and labeling, product forms, sizes and grades, and quality factors. Whereas the California Seafood Council handled distribution of the publication to its target audiences, we will distribute the publication to secondary audiences, such as the State Department of Commerce, California legislators and their aides, and others in order to educate them about the importance of the California seafood industry and provide them with information about major export products.

Public Relations and Publicity

Public relations activities designed to publicize the activities and achievements of the program take a number of forms. These include: contact with the broadcast and print media and with public affairs officers at other universities; press releases

and tip sheets; attendance at workshops, conferences, and special functions; preparation of speeches and testimony; and publication of a bimonthly newsletter, brochures, and announcements.

The Communications staff maintains a list of broadcast and print media contacts throughout the country with whom we stay in contact via mailings, tip sheets, and phone. We have participated with the national media specialist for the National Sea Grant College Program in putting together an "experts" guide, which he is distributing nationally.

We produce a bimonthly, single-page newsletter, *Sea Grant In Brief*, which we use both to maintain program visibility with a number of our key contact groups, such as government officials and industry personnel, and to inform these audiences in an encapsulated way about Sea Grant activities. This newsletter is also sent to members of the news media, who have used it for story ideas.

We also maintain photograph and slide files with which we fill media requests. These are also used in our own publications and in presentations describing our program, such as slide shows that we recently prepared for presentations to the California Seafood and Fisheries Institute.

Article Placement

A listing of newspapers and journals featuring the activities of Sea Grant scientists that resulted from our efforts includes: *Los Angeles Times, San Diego Union, MTS Currents, Santa Cruz Sentinel*, Newsletter of the California Fisheries and Seafood Institute; *University City Light* (San Diego), *San Gabriel Valley Tribune, Pacific Coast Aquaculture, The Quarterback* (U.S. Naval Postgraduate School); *Los Angeles Daily News, Contra Costa Times, West County Times, and National Fisherman.*

Also, in the last biennium, we have been successful in sponsoring and placing the following articles using freelance writers: "Sardine Survival," *Pacific Fishing*, November 1993; "Keeping Oil Spills in Check," *Pacific Fishing*, November 1993; "Management of Human Error in Operations of Marine Systems," *MTS Journal,* Spring 1994; "Quicker Answers to What Makes Us Sick," *San Diego Union,* June 1, 1994; "Inbreeding, Crossbreeding of Oyster Broodstock Under Study," *The Aquaculture News,* July 1994; "Sizing Up Coastal Dungeness Fisheries," *Pacific Fishing*, October 1994.

Speeches and Testimony

The following testimony, speeches, and slide shows were prepared for presentation by the Program Director, Dr. James Sullivan. These presentations directly support Goal 4: "To support the communications needs of program management, thereby contributing to Sea Grant's visibility and effectiveness."

- "California Sea Grant: An Excellent Investment for the State." Testimony to the Joint Legislative Committee on Fisheries and Aquaculture, Sacramento, March 1993.
- "The Sea Grant Program: A Model for These Times." Pacific Congress on Marine Science and Technology, Beijing, PRC, June 1993. (Slide Show).
- "Toxic Algae Events in the United States and Their Associated Economic and Social Impacts." Pacific Congress on Marine Science and Technology, Beijing, PRC, June 1993.
- The Case for Reauthorization of California Sea Grant." Testimony to the Senate Natural Resources and Wildlife Committee, July 1993.
- Presentation to the California Seafood and Fisheries Institute, October 1993.
- "Observations on the California Ocean Resources Management Plan." Testimony to the Assembly Select Committee on Marine Resources, La Jolla, November 1993.
- "Sea Grant's Response to Fisheries Issues in California." Testimony to the Joint Committee on Fisheries and Aquaculture, 22nd Annual Fisheries Forum, Sacramento, March 1994.
- Western Legislative Conference, Ocean Resources Committee, Sacramento, May 1994.
- Marine Biotechnology: Emerging Economic Opportunities for California," California Consortium for Marine Biotechnology, San Diego, October 1994.
- "California Sea Grant: Promoting International Cooperation in Aquaculture," Presented at the 23rd U.S.–Japan Natural Resources Symposium, Nilgata, Japan, November 1994.

National Sea Grant Programwide Activities

The following activities were performed in collaboration with colleagues in the National Sea Grant College network or in support of state and/or national program objectives.

- *"Rejuvenating the Nation's Marine Infrastructure: An Investment in Public Safety, Environmental Quality, and Economic Vitality."* The Communications Office edited, wrote sections for, and produced a 22-page proposed national research initiative in conjunction with Professor Robert Bea of the Department of Naval Architecture and Offshore Engineering at the University of California, Berkeley, and Dr. Richard Seymour of Texas A&M University. Bea and Seymour headed a Sea Grant working committee representing engineering institutions in seven states plus the National Office of Sea Grant. The document addresses the aging and technological obsolescence of much of the U.S. infrastructure of marine structures, including breakwaters, piers, pipelines, outfalls, offshore platforms and harbor structures. It identifies needed research on the reassessment and rehabilitation of marine structures and was designed to provide the basis through which the United States can better meet the engineering challenges associated with its aging marine structures. This research initiative was adopted by the Council of Sea Grant Directors and included in the National Network Plan for Sea Grant, 1995–2005.
- Prepared Congressional testimony in collaboration with Dr. Joseph Chang, CEO, OsteoArthritis Sciences, Inc. 1993.

• Wrote copy on California accomplishments in marine technology and biotechnology for network publication, *"Economic Competitiveness and the Coastal Environment."* Provided detailed critique of document prior to publication. 1993.

• Wrote and edited program copy, plus notes on speakers, for National Sea Grant Week convention, Honolulu. 1993.

• Critiqued proposed national initiative on coastal hazards drafted by North Carolina Sea Grant College Program. 1993.

• Critiqued proposed national initiative on aquaculture drafted by Virginia Sea Grant College Program. 1993.

• Wrote report of Sea Grant Accomplishments for the State Resources Agency. August 1994.

• Wrote proposal to the Environmental License Plate Fund, October 1994.

• *"History and Interactions of the California Sea Grant College and Coastal Zone Management."* Report prepared for the National Office of Sea Grant for its meeting with the Coastal Stewardship Taskforce, NOAA. August 1, 1994.

• Provided review and comments on the research and education sections of the *California Ocean Resources Management Plan* to the State Resource's Agency.

• Wrote report for NOAA Administration on how rising sea level could result in shifts of international maritime boundaries.

• Wrote report on California marine mammal issues for a meeting between the director of the Alaska Sea Grant College Program and the National Marine Fisheries Service.

• Participated on National Identity Task Force of National Sea Grant Communicators. 1994.

• Identified and interviewed women oceanographers in California for inclusion in a careers publication being prepared by Maine/New Hampshire Sea Grant College Program. 1994.

• Wrote case study of California Sea Grant accomplishments in marine pharmacology for inclusion in packet of network success stories demonstrating economic return on investment. 1994.

• Attended meetings of the Pacific Sea Grant Communicators designed to identify regional network projects, Provo, 1994.

Appendices

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Governor of California

Gray Davis
Lieutenant Governor

Curt Pringle
Speaker of the Assembly

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