CALIFORNIA SEA GRANT

Biennial Report of Completed Projects 1986–88

A Publication of the California Sea Grant College Program

CALIFORNIA
SEA GRANT

The California Sea Grant College Program is a statewide, multiuniversity program of marine research, education, and advisory services, administered by the University of California Institute of Marine Resources. Sea Grant-sponsored research contributes to the growing body of knowledge about our coastal and ocean resources and, consequently, to the solution of many marine-related problems facing our 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 for university students, public school teachers and students, and the general public so that our coastal and ocean resources can be understood and used judiciously by this and future generations.

ROSEMARY AMIDEI
COMMUNICATIONS COORDINATOR

Published by the California Sea Grant College Program, University of California, La Jolla, California, 1990. Additional single copies are available free of charge from Sea Grant at: University of California, A-032, La Jolla, California 92093-0232.

This work was sponsored in part by NOAA, National Sea Grant College Program, Department of Commerce, under grant no. NA85AA-D-SG138, Project #A/P-1 and by the California State Resources Agency. The U.S. Government is authorized to produce and distribute reprints for governmental purposes.

CALIFORNIA SEA GRANT

Biennial Report of Completed Projects 1986–88

California Sea Grant College Program
University of California
La Jolla, CA 92093
Publication No. R-CSGCP-028

TABLE OF CONTENTS

Ocean Technology

Marine Affairs

Rapid Response

Continuing Projects

Appendices

Introduction

This biennial report presents the results of research activities undertaken by the California Sea Grant College Progam during fiscal years 1986-87 and 1987-88. It is meant to be a technical record of our accomplishments for use by individuals in academia, government, and industry. This publication contains only reports of completed projects (as opposed to descriptions of work in progress). It thus forms an important historical record of program achievement.

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

California's Sea Grant College Program is administered by the University of California and is headquartered at Scripps Institution of Oceanography on the University of California, San Diego campus. The California Sea Grant Committee, composed of representatives from the University of California and State University Systems and private universities, provides administrative guidance on matters pertaining to the conduct of the Sea Grant program and the pursuit of its objectives. The committee also reviews the program subject areas and appoints independent review panels to assist it in this task.

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

James J. Sullivan
Director

Coastal Resources

Modified Watershed Hydrology: Effects on Estuarine Ecosystems

San Diego State University
R/CZ-73
Project Initiated: October 1, 1984
Project Completed: September 30, 1987

Joy B. Zedler, Ted Griswold, Abby White, and Joan Brenchley-Jackson

The principal goal of this Sea Grant project was to understand how estuarine systems respond to hydrological modifications, emphasizing increased streamflows and documenting impacts of decreased tidal influence. Both long- and short-term responses were identified by documenting ecosystem changes after prolonged discharges of reservoir water to San Diego River and Tijuana Estuary and by testing cause-effect relationships with controlled experiments (Griswold et al., 1988) and a computer simulation model (Brenchley-Jackson et al., 1988). The associated goals were to set guidelines for streamflow management that would maintain biodiversity in coastal water bodies and transfer information to resource managers throughout the region. The salt-marsh vegetation responses were emphasized because coastal wetlands are generally managed for endangered species that are dependent on specific plant communities.

Vegetation Dynamics

Salt marshes change when winter streamflows are prolonged into summer and when tidal flows are inhibited by closure of ocean inlets. The changes involve invasions of species not normally found in tidal marshes and local extinctions of native plants. We developed a low-salinity gap model to explain differential establishment of species (Figure 1; Zedler and Beare, 1986; Beare and Zedler, 1987):

During most years, intertidal marsh soils are saline to hypersaline nearly all year. Major floods create a gap in hypersalinity, whereas reservoir discharges not only reduce soil salinity but also prolong the period of the low-salinity gap. As salinity drops, more species can germinate; if low salinity persists (e.g., during periods of reservoir or wastewater discharge), exotic wetland weeds and native species from upstream marshes can become established.

The compositional changes are often persistent because after initial establishment, many of the invading species can expand vegetatively even if soils are saline or hypersaline (Beare and Zedler, 1987). For example, cattails (*Typha domingensis*) invaded the pickleweed (*Salicornia virginica*) marsh at San Diego River after a single year with reservoir drawdown (1980); they have persisted for 7 years (Figure 2). The cattail population generally declined, but frequency of occurrence held steady in 1983 when there was heavy winter rainfall and streamflow.

Additional changes in salt-marsh vegetation occur if an estuary closes to tidal flushing. If closure is followed by drought, the most broadly tolerant plant, pickleweed, persists and expands (Zedler and Nordby, 1986) as shown by field data and supported by experiments performed with the simulation model (Figure 3). Other species (e.g., the short-lived plants *Salicornia bigelovii* and *Suaeda esteroa*) decline or die out from drought and hypersalinity. If runoff is impounded during closure, prolonged inundation stresses pickleweed (Figure 4) and bare spaces develop in the canopy. Although pickleweed is capable of rapid recovery through seed germination and seedling establishment as soon as tidal flushing resumes, other species may not be, because their seeds require reduced salinity to germinate (Zedler and Beare, 1986). Thus, the result of even infrequent closure is reduced species diversity, with pickleweed as the most common dominant. Cordgrass (*Spartina foliosa*) has limited resilience, in part because seedlings do not establish readily without freshwater flooding and in part because pickleweed is a superior competitor.

Competitive Interactions

Cordgrass and pickleweed occupy quite similar habitats, and population changes are often reciprocal. The 1984 nontidal drought killed large areas of cordgrass at Tijuana Estuary, whereas pickleweed expanded its distribution to fill gaps in the canopy (Zedler and Nordby, 1986). Cordgrass has not yet recovered its pre-1984 distribution (Figure 5). In 1987, we tested the

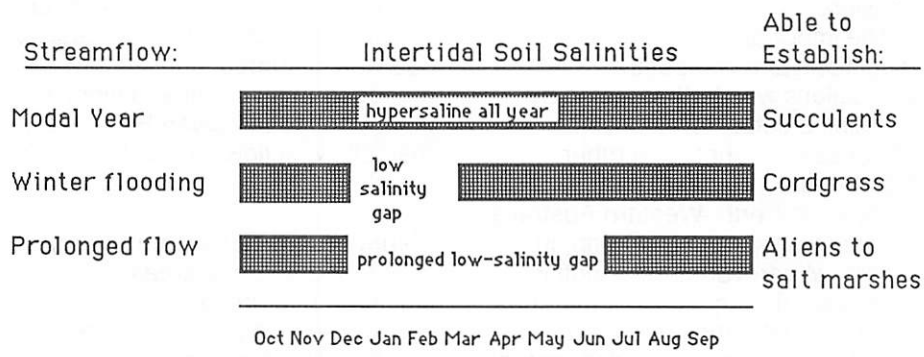


Figure 1. The "low-salinity gap" model explains differential establishment of plant species in modal years (with limited winter streamflow), with winter flooding, and with streamflows artificially prolonged by reservoir or wastewater discharges.

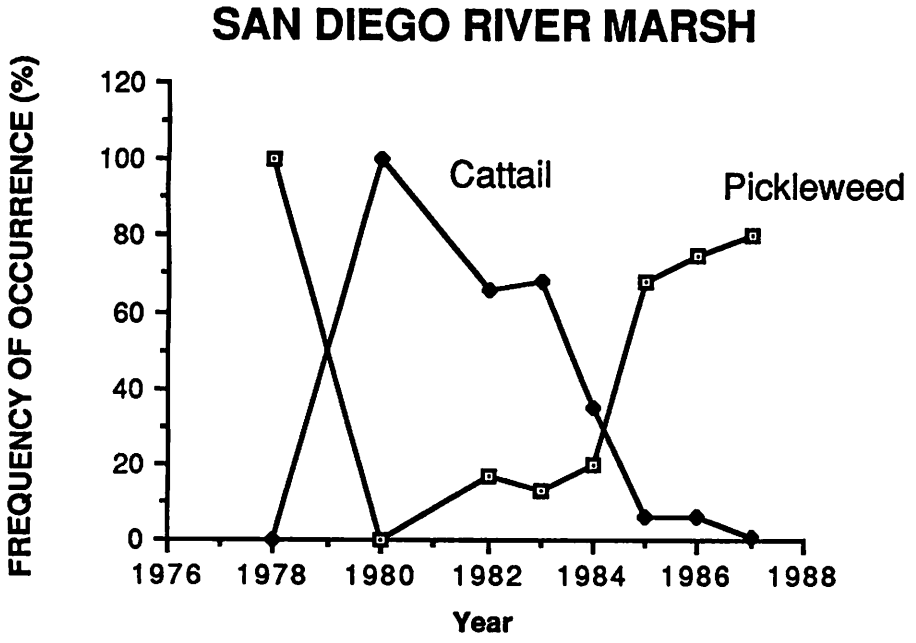


Figure 2. Changes in the distribution of salt marsh dominants at the San Diego River Marsh. Cattails replaced pickleweed during the 1980 flood and prolonged reservoir discharge period; cattails have since declined, although the 1983 flood year temporarily revitalized the population; 1% of the 250 monitoring stations retain cattails.

hypothesis that recovery was hindered by pickleweed competition. In field experiments (Figure 6), cordgrass showed a competitive release response, which was consistent with earlier results (Covin, 1984; Covin and Zedler, 1988) of experiments done under more sluggish tidal flows. Simulation modeling to incorporate competitive interactions is being continued by Joan Brenchley-Jackson and Gerrit Platenkamp, with funding from NOAA OCRM Marine and Estuarine Management Division.

The importance of salinity reductions and competitive interactions was further tested by examining cattail invasions into native salt marshes in another Mediterranean-type estuary, adjacent to Perth, Western Australia (Zedler, Paling, and McComb, in press). Wherever street runoff is channeled through the salt marshes, an exotic to the region, *Typha orientalis*, invades areas dominated by the native *Juncus krausii*. Experiments with seeds, seedlings, and rhizome-bearing plants of both species supported the hypotheses developed in San Diego County. The native species had higher salt tolerance than the invader. When low winter soil salinities are prolonged (by urban runoff) well beyond the usual period, alien plants germinate, seedlings develop rhizomes, and vegetative reproduction allows persistence. However, under the experimental conditions, the cattail expanded its distribution only under low salinities and was competitive only as an adult, rhizome-bearing plant. This suggested that establishment of seedlings would occur mainly near street drains, where native marsh sod was disturbed, and led to management recommendations to pipe runoff to the Swan River, rather than allowing flows through the salt marsh.

Management Recommendations

At present, urban areas are clustered near the ocean, and wastewater is piped to deep ocean outfalls. As development progresses inland, existing sewage plants become overloaded, and the cost of piping effluent to the ocean increases. Thus, several proposals have been made to treat wastewater locally for release to coastal streams and eventual flow into estuaries. To maintain habitat for endangered species, it is essential that salt marshes be managed to prevent compositional shifts in both canopy vegetation and food chains. Modifications in hydrology must therefore be kept within the natural range of salinity and streamflow regimes. We make the following recommendations:

Streamflows must be managed to prevent prolonged reductions in salinity in estuarine waters and soils. Results indicate that the safe threshhold for preventing species invasions is maintenance of salinities above 30 ppt for 7 to 8 months of the year. Establishment and persistence are likely if salinities drop to 20 ppt for 4 to 5 months. Thus, to reduce risks of vegetation shifts, we recommend that streamflows not reduce salinities below 30 ppt for more than 4 months and not reduce salinities during the normal dry season (April through September). Marine salinities should be maintained during the dry season by managing for continual tidal flushing. This will simultaneously insure that soils are neither inundated for prolonged periods of time (which damages *S. virginica*) nor subjected to prolonged drought (which damages *S. foliosa*).

In general, augmented streamflows will cause fewer problems if restricted to winter; the greatest impacts result from prolonging winter flows through summer. Salinities lower than 20 ppt will have less effect if restricted to short periods of time. With a brief, rather than prolonged, reduction in salinity, the main species affected would be invertebrates and fishes. Reduced salinities will have less impact if changes occur slowly; this would reduce shocks to estuarine animals.

Estuaries with large tidal prisms and small watersheds will have greater tolerance for increased streamflows. Lagoons that close to tidal flushing are most susceptible to increased freshwater influence. Impacts will be seen first as

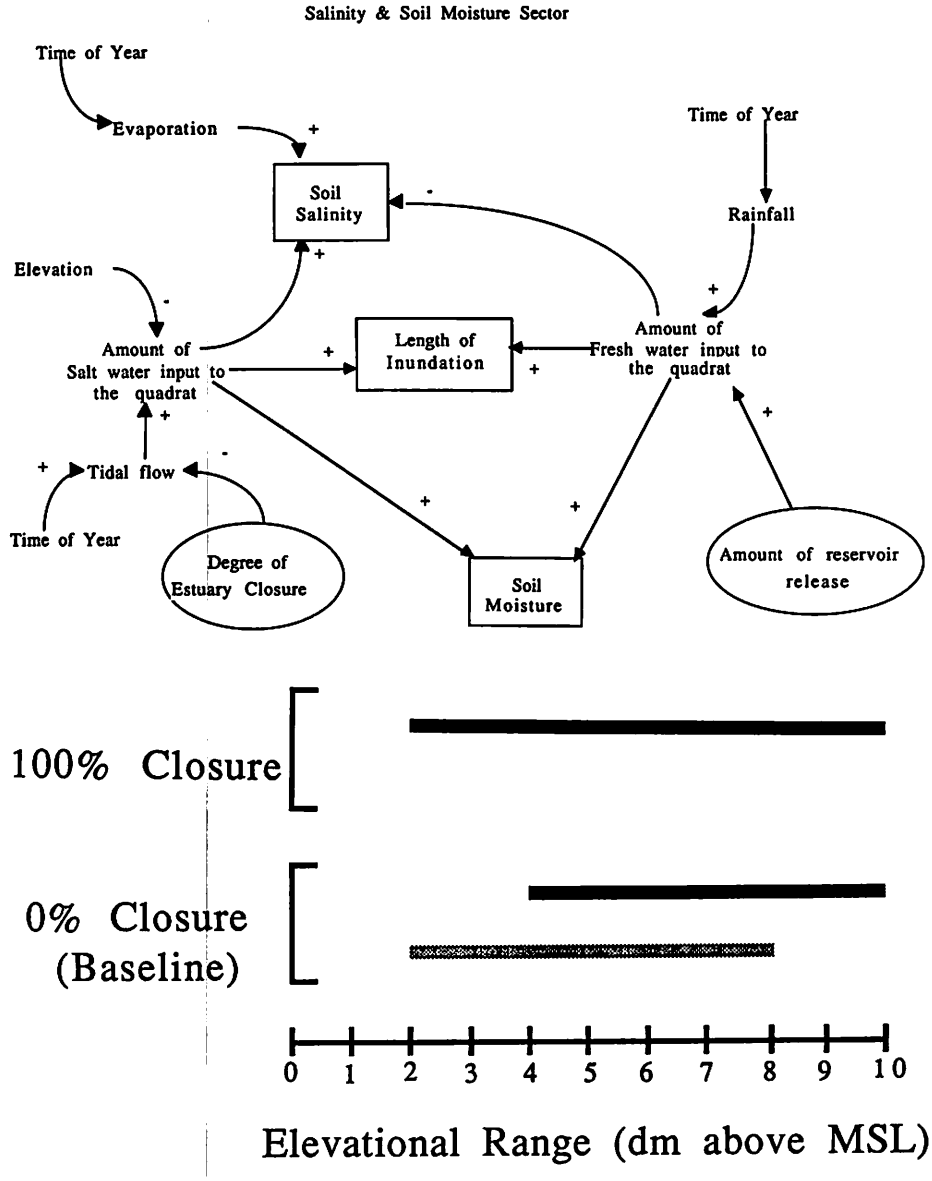


Figure 3. Factors influencing salinity and soil moisture are shown in a conceptual presentation of one part of the simulation model (Brenchley-Jackson et al., in press). Computer simulations indicate changes in both cordgrass (stippled bar) and pickleweed (solid bars) with different degrees of closure to tidal flushing.

mortality of invertebrate and fish populations, and later as invasions of brackish marsh vegetation.

Because invasions of plant species are facilitated by soil disturbances and heavy sedimentation, we recommend the following: (1) Urban runoff should not be channeled into wetlands, and sediment traps should be added to areas where street drains are unavoidable. Such traps will require continual maintenance with irrigation runoff, as well as immediate clean-out following rainfall events. (2) Any landscaping adjacent to coastal marshes should use native species that do not require irrigation. Plants should be cultured and planted in native soil, rather than greenhouse soil mix, to reduce chances of stimulating invasion of exotic species. (3) Restoration of off-road vehicle paths (which are often present in upper salt marshes) should not include widescale disking, which encourages the invasion of salt-tolerant exotics. Instead, the reinvasion of native plants should be encouraged by fencing to terminate trampling and by hand planting during the winter wet season.

Because augmented streamflows can change salt-marsh vegetation, alternatives to discharging treated wastewater to coastal streams must be sought. In decreasing order of preference, we recommend total recycling (once removal of pathogens is assured), reuse in irrigation and groundwater recharge, and impoundment to create artificial wetlands. These measures will not only protect coastal wetlands but also reduce demands for imported water. Artificial wetland impoundments can be created to consume treated wastewater (through transpiration), as well as to aid in the treatment process. Artificial wetlands require land area and must be managed to prevent mosquito problems, but they provide benefits to wildlife and can have educational and esthetic values. Because evaporation rates are high (approximately 2 m/yr), summer discharges could be substantially reduced by diverting wastewater effluent to constructed wetlands. If discharges to coastal streams are unavoidable, then opportunities to use wastewater to enhance degraded riparian habitats should be identified within the affected stream.

Modifications to tidal flow are also of concern, because reduced tidal flushing leads to wider fluctuations in estuarine or lagoon salinity, water levels, temperature, dissolved oxygen, and other attributes. Maintenance of populations of native species requires that tidal flushing be maintained in late summer, when physiological stress due to high temperatures may be greatest. In early spring, when species invasions are likely and native plants experience exponential growth, maintenance of tidal flushing is also critical if brackish or fresh water has been impounded. The low salinities will allow alien plants to invade, and high water levels will kill native species. At the same time, marine fishes and invertebrates will suffer from lack of access to the lagoon or from osmotic stress if trapped within the lagoon.

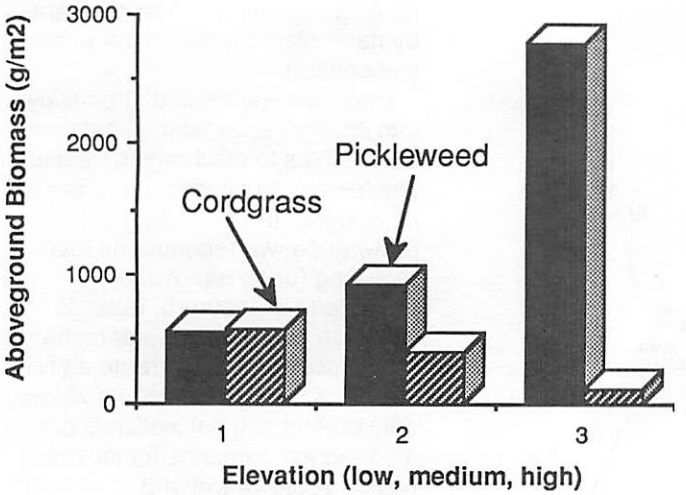


Figure 4. Growth of cordgrass and pickleweed at three elevations in mesocosms at PERL (Griswold, 1988).

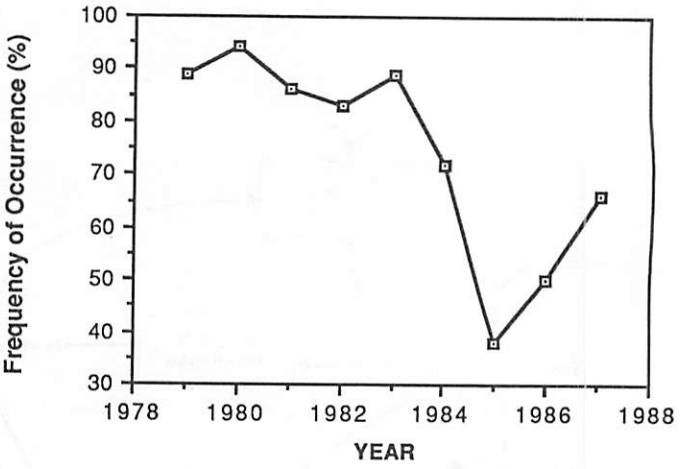


Figure 5. Cordgrass declined in the 102 monitoring stations at Tijuana Estuary following the 1984 hypersaline drought when the ocean inlet was closed. The population has since expanded but not recovered to pre-drought conditions.

Specific limits on streamflow volumes must be developed for individual types of stream-estuary systems, by using the biological limits developed from field and experimental studies and summarized in the simulation model. Engineering hydrologists can now develop predictions for site-specific soil salinities, given the estuary's tidal prism, tidal levels near the estuary, measurements of tidal lags and damping effects in major tidal channels, data on historic streamflows entering the estuary, streamflow-salinity relationships or data on mixing characteristics for the estuary, and water-soil salinity relationships.

These recommendations have been transferred to agency personnel who have requested site-specific advice. Throughout the study, frequent interactions have occurred with federal, state, and local wetlands managers.

Conclusion

Hydrological modifications that prolong the period of freshwater influence, either by extending winter streamflows or reducing tidal influence, can cause shifts in vegetation that reduce estuarine wetland values for native fauna, including endangered species. This study identified the patterns of change and their cause-effect relationships, produced a simulation model that predicts changes in the habitat of endangered species after alterations of salinity or inundation, and provided recommendations for management.

The ecological significance of the responses of vegetation to altered hydrology is that one-time modifications can have long-term effects on the ecosystem structure. The importance to management is that the biodiversity of estuarine salt marshes is best maintained under the natural hydrologic regime (i.e., continual tidal flushing with winter streamflows, including occasional floods).

Cooperating Organizations

City of San Diego
California Coastal Commission
California Department of Parks and Recreation
California Resources Agency
California State Coastal Conservancy
Management Authority, Tijuana River National Estuarine Research Reserve
NOAA Office of Ocean and Coastal Resources Management, Marine and Estuarine Management Division
U.S. Fish and Wildlife Service

References

Beare, P. A. 1984. Salinity tolerance in cattails (*Typha domingensis* Pers.): Explanations for invasion and persistence in a coastal salt marsh. Master's thesis, San Diego State University.

Beare, P. A., and J. B. Zedler. 1987. Cattail invasion and persistence in a coastal salt marsh: The role of salinity reduction. *Estuaries* 10(2):165–170.

Brenchley-Jackson, J. L., T. C. Foin, and J. B. Zedler. 1988. Modeling the effect of salinity and inundation on *Spartina* and *Salicornia* in Southern California. In: *Wetland and Riparian Ecosystems of the American West.* K. M. Mutz and L. C. Lee, eds. Proceedings, Eighth Annual Meeting of Society of Wetland Scientists, May 1987, Seattle, Washington. pp. 261–266.

Covin, J. D. 1984. The role of inorganic nitrogen in the growth and distribution of *Spartina foliosa* at Tijuana Estuary, California. Master's thesis, San Diego State University.

Covin, J. D., and J. B. Zedler. 1988. Nitrogen effects on *Spartina foliosa* and *Salicornia virginica* in the salt marsh at Tijuana Estuary, California. *Wetlands* 8:51–65.

Griswold, T. J. 1988. Physical factors and competitive interactions affecting saltmarsh vegetation. Master's thesis, San Diego State University.

Griswold, T. J., C. Nordby, and J. B. Zedler. 1988. Simulated salt marshes: Methods for reducing experimental error. In: *Wetland and Riparian Ecosystems of the American West.* K. M. Mutz and L. C. Lee, eds. Proceedings, Eighth Annual Meeting, Society of Wetland Scientists, May 1987 Seattle, Washington. pp. 315–317.

Onuf, C. P., and J. B. Zedler. 1988. Pattern and process in arid-region salt marshes—southern California. In *The ecology and management of wetlands.* D. D. Hook, et al., eds.

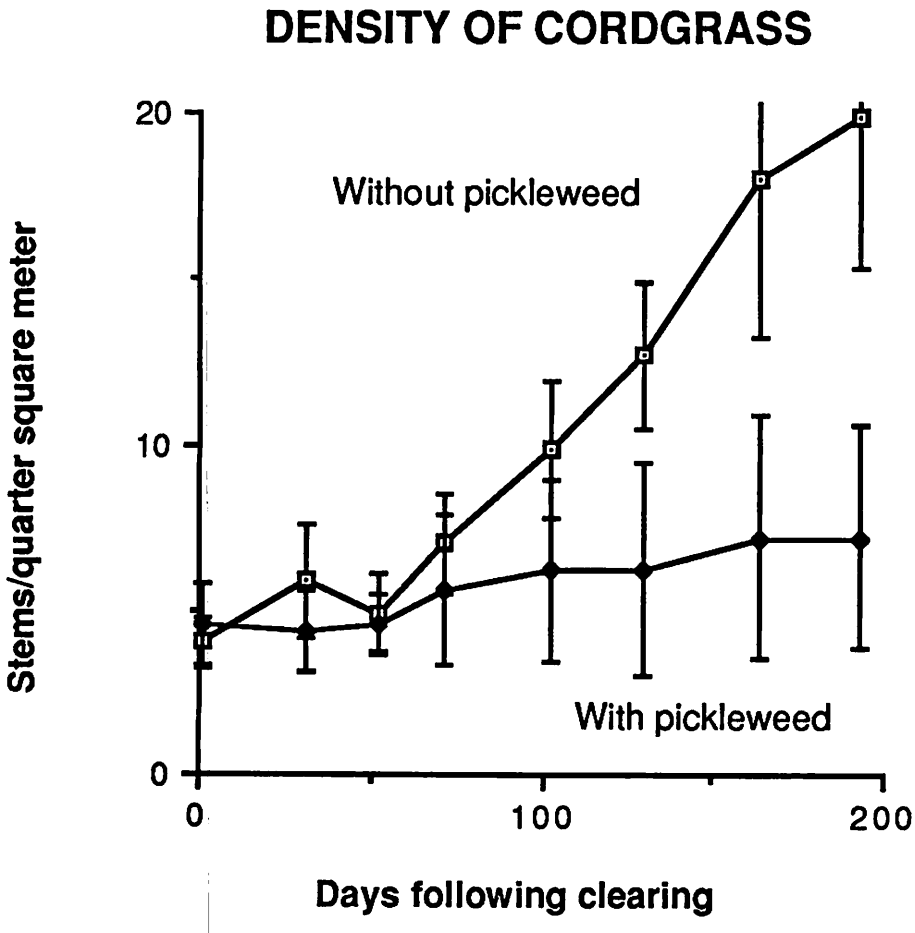


Figure 6. Field experiments at Tijuana Estuary demonstrated that the expansion of cordgrass in 1987 was limited by the presence of pickleweed. Data are from replicate square-meter plots within areas formerly dominated by cordgrass (Griswold, 1988).

Croom Helm, London, U.K. pp. 570–581.

White, A. 1986. The effects of habitat type and human disturbance on an endangered wetland bird: Belding's Savannah sparrow. Master's thesis, San Diego State University.

Zedler, J. B. 1986. Catastrophic flooding and distributional patterns of Pacific cordgrass (*Spartina foliosa*). *Bull. South Calif. Acad. Sci.* 85:74–86.

Zedler, J. B., and P. Beare. 1986. Temporal variability of salt marsh vegetation: The role of low-salinity gaps and environmental stress. In *Estuarine variability*. D. A. Wolfe, ed. Academic Press, New York. pp. 295-306.

Zedler, J. B., J. D. Covin, C. Nordby, P. Williams, and J. Boland. 1986. Catastrophic events reveal the dynamic nature of salt-marsh vegetation in southern California. *Estuaries* 9:75–80.

Zedler, J. B., and C. S. Nordby. 1986. *The ecology of Tijuana Estuary: An estuarine profile*. U.S. Fish and Wildlife Service Biol. Rep. 85(7.5). Washington, D.C. 104 pp.

Zedler, J. B., and C. P. Onuf. 1984. Biological and physical filtering in arid-region estuaries: Seasonality, extreme events, and effects of watershed modification. In *The Estuary as a Filter*. V. S. Kennedy, ed. Academic Press, New York. pp. 415–432.

Zedler, J. B., E. Paling, and A. McComb. In press. Differential salinity responses help explain the replacement of native *Juncus kransii* by *Typha orientalis* in western Australian salt marshes. *Australian J. Ecology*.

Publications

Beare, P. A. 1984. Salinity tolerance in cattails (*Typha domingensis* Pers.): Explanations for invasion and persistence in a coastal salt marsh. Master's thesis, San Diego State University.

Covin, J. D. 1984. The role of inorganic nitrogen in the growth and distribution of *Spartina foliosa* at Tijuana Estuary, California. Master's thesis, San Diego State University.

Beare, P. A., and J. B. Zedler. 1987. Cattail invasion and persistence in a coastal salt marsh: The role of salinity reduction. *Estuaries* 10(2):165–170.

Brenchley-Jackson, J. L., T. C. Foin, and J. B. Zedler. 1988. Modeling the effect of salinity and inundation on *Spartina* and *Salicornia* in Southern California. In: *Wetland and Riparian Ecosystems of the American West*. K. M. Mutz and L. C. Lee, eds. Proceedings, Eighth Annual Meeting of Society of Wetland Scientists, May 1987, Seattle, Washington.

Covin, J. D., and J. B. Zedler. 1988. Nitrogen effects on *Spartina foliosa* and *Salicornia virginica* in the salt marsh at Tijuana Estuary, California. *Wetlands* 8:51–65.

Griswold, T. J. 1988. Physical factors and competitive interactions affecting saltmarsh vegetation. Master's thesis, San Diego State University.

Griswold, T. J., C. Nordby, and J. B. Zedler. 1988. Simulated salt marshes: Methods for reducing experimental error. Proceedings, Eighth Annual Meeting, Society of Wetland Scientists, May 1987 Seattle, Washington.

Onuf, C. P., and J. B. Zedler. 1988. Pattern and process in arid-region salt marshes—southern California. In *The ecology and management of wetlands*. D. D. Hook, ed. Timber Press, Portland, Oregon.

White, A. 1986. The effects of habitat type and human disturbance on an endangered wetland bird: Belding's Savannah sparrow. Master's thesis, San Diego State University.

Zedler, J. B. 1986. Catastrophic flooding and distributional patterns of Pacific cordgrass (*Spartina foliosa*). *Bull. South Calif. Acad. Sci.* 85:74–86.

Zedler, J. B. 1986. Wetland restoration: Trials and errors or ecotechnology? In *Wetland functions, rehabilitation, and creation in the Pacific Northwest: The state of our understanding*. R. Strickland, ed. Washington State Department of Ecology, Olympia, Washington, April 1986.

Zedler, J. B. 1988. Restoring diversity in salt marshes: Can we do it? In *Biodiversity*. E.O. Wilson, ed. National Academy Press, Washington, D.C. pp. 317–325.

Zedler, J. B. 1988. Salt marsh restoration: Lessons from California. In *Rehabilitating Damaged*

Ecosystems. J. Cairns, ed. CRC Press, Boca Raton, Florida. pp. 123–138.
Zedler, J. B., and P. Beare. 1986. Temporal variability of salt marsh vegetation: The role of low-salinity gaps and environmental stress. In *Estuarine variability.* D. A. Wolfe, ed. Academic Press, New York. pp. 295-306.
Zedler, J. B., J. D. Covin, C. Nordby, P. Williams, and J. Boland. 1986. Catastrophic events reveal the dynamic nature of salt-marsh vegetation in southern California. *Estuaries* 9:75–80.
Zedler, J. B., and C. S. Nordby. 1986. *The ecology of Tijuana Estuary: An estuarine profile.* U.S. Fish and Wildlife Service Biol. Rep. 85(7.5). Washington, D.C. 104 pp.
Zedler, J. B., and C. P. Onuf. 1984. Biological and physical filtering in arid-region estuaries: Seasonality, extreme events, and effects of watershed modification. In *The Estuary as a Filter.* V. S. Kennedy, ed. Academic Press, New York. pp. 415–432.
Zedler, J. B., E. Paling, and A. McComb. In press. Differential salinity responses help explain the replacement of native *Juncus kransii* by *Typha orientalis* in western Australian salt marshes. *Australian J. Ecology.*

Lectures and Conferences

Zedler, J. B., and S. E. Ibarra-Obando. Impacts of hydrological modifications on Pacific coastal water bodies. Invited paper, Border Water Resources Symposium, Pacific Divison of the American Association for the Advancement of Science, San Diego, June 1987.
Zedler, J. B. Species invasions: The role of extreme events. Invited talk, International Botanical Congress, Berlin, West Germany, July 1987.
Zedler, J. B. Has coastal planning worked? An ecologist's view. Invited talk, California Chapter, American Planning Association, San Diego, August 1987.
Zedler, J. B. Why it's so difficult to replace lost wetland functions. Invited talk, National Wildlife Federation symposium, Increasing Our Wetland Resources, Washington, D.C. October 1987.

Study of Extreme Coastal Sea Level

Daniel R. Cayan and Reinhard E. Flick

University of California, San Diego
California Department of Boating & Waterways
Scripps Institution of Oceanography
R/CZ-76
Project Initiated: October 1, 1986
Project Completed: September 30, 1988

Much of the previous work done on the study of storm surge has dealt with the large, highly destructive surges found in broad, shallow areas such as the continental shelf regions of the Bay of Bengal, the North Sea, and the Gulf of Mexico. In those areas and many others, storm surge can play a dominant role in coastal flooding. But often it is the coincidence of storm surge with high tides and other oceanographic factors that generates coastal flooding.

It was the simultaneous occurrence of several oceanographic and meteorological factors that caused the extensive coastal floods of the winter of 1982–1983 along the California coast (damage estimates totaled more than $100 million). In addition to wave attack (Seymour et al. 1984), Cayan and Flick (1985) identified four of these factors: high predicted astronomical tide, storm surge due to strong North Pacific storms; high sea level associated with the El Niño event of 1982–1983, and the cumulative effect of slow, secular rise in sea level. This study focuses mainly on the effects of meteorological forcing in producing high sea levels along the southern California coast.

The winter of 1982–1983 was marked by several extreme sea-level episodes (Flick and Cyan, 1985). These were forced by strong North Pacific storms and aggravated by generally higher sea levels over the period ascribed to broad-scale warming of the Eastern Pacific surface waters associated with the unusually strong El Niño event during this period. Statistical analysis of the sea-level anomaly, wind field, atmospheric pressure field, and the sea-surface temperature along with the examination of five of these extreme sea-level events provides insight into the generation of sea-level fluctuations by meteorological and oceanographic forcing.

It is interesting that the damage that occurred in a more recent very severe storm that hit the Southern California Coast in January 1988 was not as bad as it could have been had all of the above-mentioned factors been operating. Although the meteorological influences of wind and sea level pressure produced exceptionally high waves and storm surge (Strange et al. 1989; Flick and Badan-Danyon 1989), peak tide during the storm was only moderately high and background sea level anomalies were slightly lower than normal.

Hourly sea-level data were collected for two coastal locations and one offshore island station within the Southern California Bight region and covering winter 1982 through summer 1983. The coastal

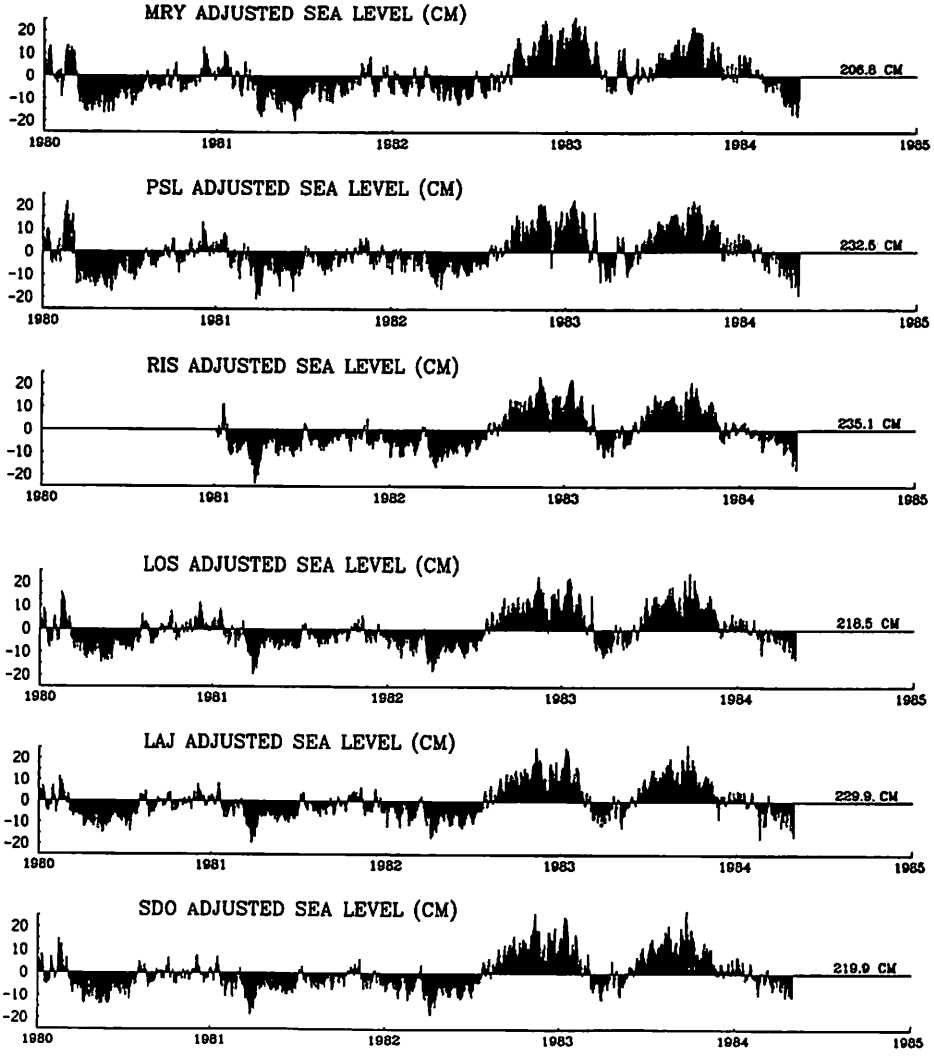


Figure 1. Sea level anomaly at six Southern California stations (Monterey, Port San Luis, Rincon Island, Los Angeles, La Jolla, and San Diego) during 1980-1984. Sea level has been filtered to remove the tide and adjusted to remove atmospheric pressure effects.

station's sea levels were taken from Newport Beach and La Jolla, and offshore sea level was taken from Santa Catalina Island. A multiple-input linear statistical analysis (Flick, 1986; Fu, 1988) revealed that local meteorological forcing and sea-surface temperature could explain 80% of the variance of the anomalous sea level during this period. Results of the analysis also showed that the inverse barometer response is quite accurate for the Southern California Bight, although the sea surface does not exactly respond as an inverse barometer to atmospheric pressure forcing. Surface pressure forcing was the most important forcing factor of sea-level fluctuations in the frequency range 0.8000 cycles/day to 0.0333 cycles/day (1.2- to 30-day periods).

Thermal adjustments of the water column as indicated by the sea-surface temperature also played a role in forcing the variations in sea level. For the 1982–1983 winter, sea-surface temperature variations explained up to 10% of the adjusted (inverse barometer effect removed) sea-level variance and may be relatively more important at longer periods, shown by several years of daily mean adjusted sea level (Figure 1). On average during later 1982 and throughout 1983, Southern California adjusted sea levels stood about 10 cm above their mean level due to the aggregate El Niño effect.

Local meteorological forcing plays a major role in influencing sea-level fluctuations in the frequency range of 0.8000 cycles/day to 0.0333 cycles/day. In winter months, when storms are common, approximately 80% of the variance can be explained by the local atmospheric pressure, wind field, and sea-surface temperature. In summer, the skill of the estimate drops to 45% at the coast and 60% at Santa Catalina. The remaining adjusted sea-level variance is most likely due to distant meteorologically generated sea-level fluctuations that propagate into the bight from the south as coastally trapped waves.

The episode between February 28 and March 5, 1983, was the strongest southern California storm event of the 1982–1983 winter. A sequence of weather maps are shown in Figure 2. The surface atmospheric pressure drop was more than 17 millibars with wind speeds of more than 40 km/hr. Compared with the earlier storms, the storm path through the Southern California Bight was more southerly displaced, and the duration of the storm was longer. As it had in previous storms, the peak in west-to-east wind stress led the peak in long-shore wind stress, and both led peak positive sea-level anomalies. Adjusted sea-level anomalies reached 5 cm, with total sea-level anomalies (as shown in Figure 3) exceeding 15 cm. As the storm passed to the east, the west-to-east winds reversed, and the longshore wind swung around to north to south. The offshore wind stress peak was followed 30 hours later by a drop in sea level, generating an increase in sea level at the coast 20 hours later. The time lag for the offshore wind stress corresponds with the values found in the statistical analysis for the entire winter period. The time lag for longshore wind stress, however, was as much as twice as long as the statistical time lag, probably because of the unusual duration of the strong winds, the size of the storm, and the storm's path and speed. The storm also seemed to stall for about a day before moving through the bight.

Over the entire winter, most of the

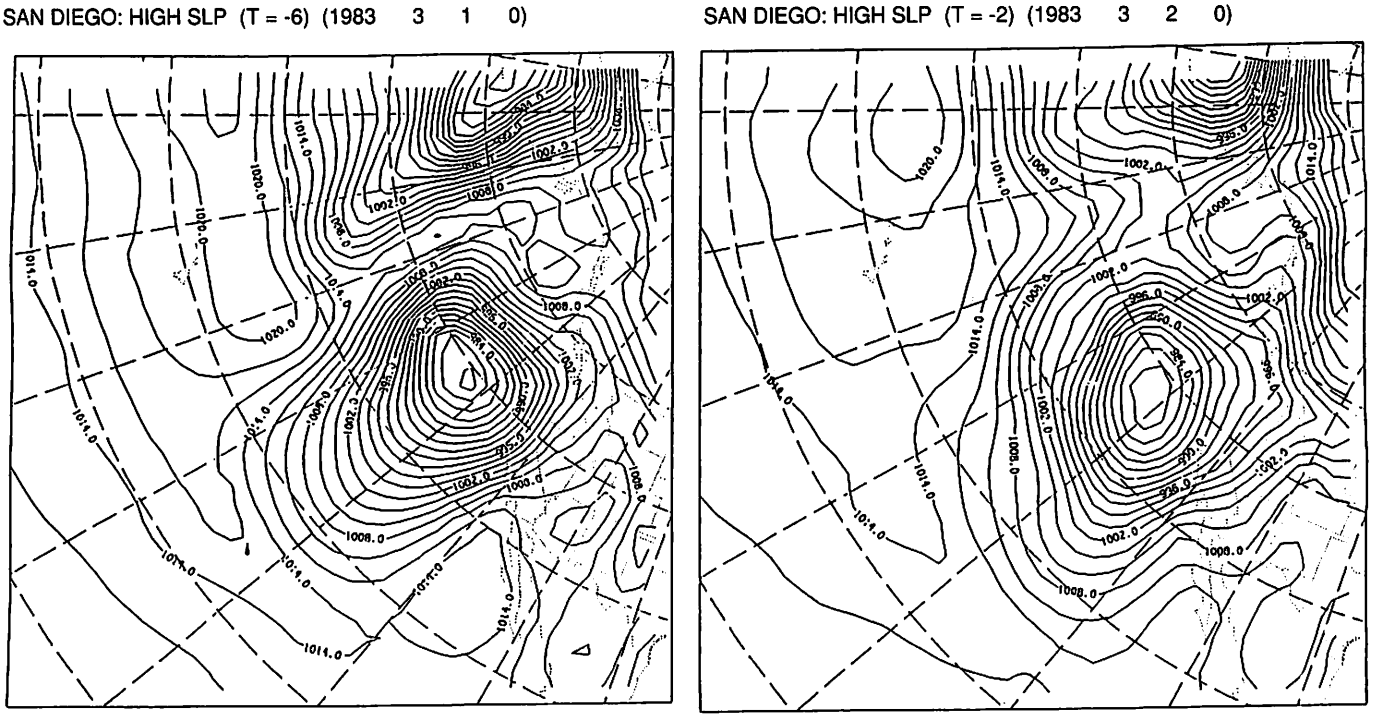


Figure 2. Atmospheric sea level pressure over eastern North Pacific during March 1-3, 1983. Contour interval 2 mb.

extreme storm events displayed peaks in both cross-shore and longshore wind stress. In the five storms examined form 1982 to 1983, the cross-shore wind stress peaks led the longshore peaks by 15 to 20 hours. Sea-surface response to the wind stress has a greater influence on sea-level variability at 1- to 30-day time scales. This was generally true, though cross-shore wind stress effects were also seen in the sea-level record. The correlation analysis of the wind fields showed that wind stress could explain about 18% of the variance of the adjusted anomalous sea-level record in the winter when storms were prevalent. Results for the summer showed only an 8% skill in estimating the adjusted sea-level anomaly.

Both statistical analysis and direct examination of meteorological and sea-level observations revealed that storm duration, the speed at which the storm traveled, and storm path all heavily influence how effective the storm-associated winds are in causing sea-level fluctuations. The spatial scale of the sea-surface response to atmospheric pressure and wind was shown to be larger than the distance between the sea-level stations. This was seen in the results of the statistical analysis for Santa Catalina and Newport Beach as well as from examination of the sea-level record for those two stations.

The results provided by this study are somewhat tentative, because of the relatively short time series (winter of 1982–1983 and the summer of 1983) available at Santa Catalina Island, allowing examination of only a few storm events. This is reflected by the artificial skill of the estimates, which were approximately 0.020 for the winter of 1982–1983 and 0.04 for the summer of 1983.

Further investigations will be able to better examine the cross-shelf spatial scale of the sea-level response of sea-level data at more offshore locations. The geostrophic current can then be estimated at several locations throughout the bight. This will allow for a more accurate estimate and a more complete picture of the current. Directly observed velocity measurements within the bight would be of great use to verify these estimated currents from sea level. A more complete study of the sea-level variability would also try to include remotely forced sea-level fluctuations. This could not be done in the present study because of the lack of sea-level and meteorological data from a station south of the bight.

Processing and analysis of long historical records of hourly sea level was started under the present project. Records from San Francisco (1900–1984) and La Jolla (1955–1984) are available and have been examined by removing the predicted tide. Statistical analysis of these anomaly records has been slowed, however, because of the many unanticipated jumps, gaps, and timing errors in the data. Methods for removing the bad data and filling gaps are currently being developed. This clean-up work is now nearly complete, and next the recurrence intervals of sea levels above the predicted tides will be calculated. New methods have recently been developed to rationally compute the joint probability densities of high tides and storm surge (Tawn, 1988). Application of these methods to the San Francisco and La Jolla time series will give coastal engineers greatly improved estimates of the probability of exceeding given sea-level heights.

SAN DIEGO: HIGH SLP (T = 2) (1983 3 3 0)

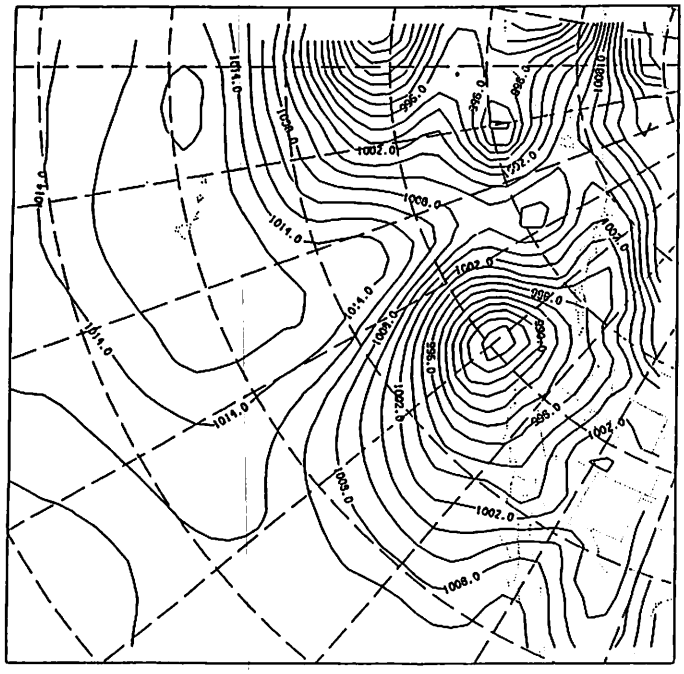


Figure 2. (Cont'd.)

Cooperating Organizations

California Department of Boating and Waterways

References

Cayan, D. R. and R. E. Flick. 1985. Extreme sea levels in San Diego, California. Scripps Institution of Oceanography Reference Series #85-3.

Flick, R. E., 1986. A review of conditions associated with high sea levels in Southern California. *Sci. Tot. Envir.* 55:251–259.

Flick, R. E. and A. Badan-Dangon. 1989. Coastal sea levels during the January 1988 storm off the Californias. *Shore and Beach* 57:28–31.

Flick, R. E., and D. R. Cayan. 1985. Extreme sea levels on the coast of California. *Proceedings of the 19th Coastal Engineering Conference ASCE,* September 1984, Houston, Texas. pp. 886–898.

Fu, T. C. Y. 1988. Southern California Bight sea level response to local atmospheric forcing. Master's thesis,

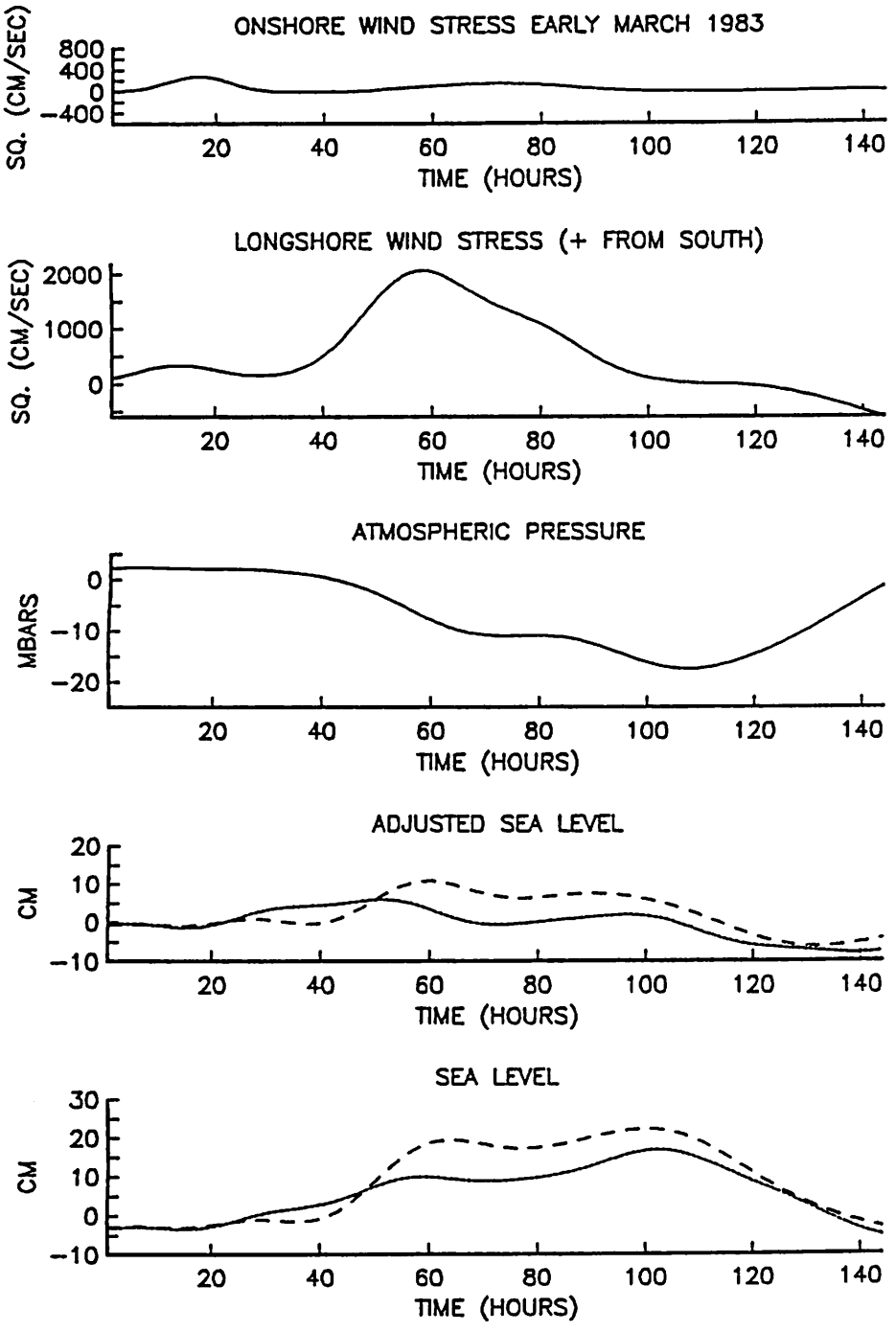


Figure 3. Typical storm time series. Early March 1983. (a) Cross-shore wind stress (+ from East), (b) Longshore wind stress (+ from South), (c) Atmospheric pressure, (d) Adjusted sea level, (e) Sea level. Small dash–Catalina, large dash–Newport.

University of California, San Diego. 155 pp.

Seymour, R. J., R. R. Strange, D. R. Cayan, and R. A. Nathan. 1984. Influence of El Niños on California's wave climate. *Proceedings 19th International Conference for Coastal Engineering. Amererican Society of Civil Engineers.* pp. 577–592.

Strange, R. R., Graham, N. E., and D. R. Cayan. 1989. Meteorological development of the unusually severe Southern California coastal storm during January 16–18, 1988. *Shore and Beach* 57:3–9.

Tawn, J. A. 1988. An extreme-value theory model for dependent observations. *J. of Hydrology* 101:227-250.

Aquaculture

Control of Reproduction in Crustaceans

University of California, Riverside
R/A-59
Project Initiated: October 1, 1984
Project Completed: September 30, 1988

Prudence Talbot

Our main objectives during the past 4 years have been to (1) refine our procedure for artificial insemination of lobsters, (2) determine if banked lobster sperm are fertile, (3) analyze and control the mechanism of egg attachment in lobsters, (4) investigate the role of the pleopod tegumental glands (cement glands) in lobster egg attachment, (5) examine the functional morphology of the reproductive tract in male shrimp, and (6) examine the morphological changes occurring in the male shrimp reproductive tract in captivity. Our progress in each of these areas will be summarized separately.

Artificial Insemination

We were successful in developing a simple technique for artificially inseminating freshly molted lobsters (Talbot et al., 1986). This technique has been used by P. Wilson of Aquaculture Enterprises, who was instrumental in providing many of the resources needed for this aspect of the project. We noted early in the project that the artificially inseminated spermatophores were not surrounded in the thelycum by a hard plug as is characteristic of naturally mated females. However, this did not seem to affect fertility; thus we focused our attention on other projects.

Fertility of Banked Sperm

We completed development of a technique for the long-term storage of lobster spermatophores (Ishida et al., 1986). Numerous freshly molted female lobsters were artificially inseminated with banked lobster sperm by J. Spague at Aquaculture Enterprises. Most of these females did not spawn or spawned and did not attach their eggs well. These are common problems with captive female lobsters. We did note that at least one of the inseminated females produced viable embryos, thus establishing that the banked sperm are fertile. We would have collected more data on banked spermatophores, but given the problems with egg attachment, this was not realistic. We therefore focused our attention on the more immediate problem of egg attachment.

The Mechanism of Egg Attachment

In collaboration with P. Wilson, we established that there is a problem with attachment and retention of eggs by captive female lobsters (Talbot et al., 1984). We subsequently deduced that epibiotic bacteria are not causative, unless their numbers become excessively high, and that they may in fact be protective (Harper and Talbot, 1984). We then showed that a major factor in poor egg attachment/retention was improper formation of the attachment stalk (Talbot and Harper, 1984). In wild females, this stalk is thick and opaque. In many captive females, the stalk is thin and fragile. This prompted us to examine the formation of the egg coat and the mechanism of egg attachment; these topics had been controversial for many years.

In collaboration with Professor Marie Goudeau of France, we showed that the only coat around the spawned lobster egg is formed by the follicle cells while the egg is in the ovary. After fertilization, a complex cortical reaction results in the formation of a second coat between the egg's surface and first coat. The two coats combine to form the fertilization envelope (Talbot and Goudeau, 1988). We then showed that the outer coat becomes deflected off the egg's surface to form the attachment stalk that wraps around the ovigerous setae of the pleopods (Goudeau et al., 1987). These observations are consistent with the idea that in captivity the reproductive cycle of the female is often intentionally accelerated, and this seems to allow insufficient time for the egg coats to form. As a result, at spawning there is insufficient coat material, and attachment stalks are undersized. These observations had an important impact on P. Wilson's program. He has moved his brood-stock females to Hawaii, where he has access to cold water. He will be able to maintain them at natural water temperatures and, it is hoped, achieve full maturation of the egg coats in the ovary. This should eliminate the major problem accounting for poor egg attachment and loss in captive females.

My graduate student, David Howard, has developed an *in vitro* system for examining the contractile properties of the lobster ovary. He has made many novel observations on this tissue, and his work may eventually lead to a better understanding of spawning and its control.

The Role of Pleopod Tegumental Glands in Egg Attachment

We have completed an extensive study on the functional morphology of pleopod tegumental glands (cement glands) in the lobster (Johnson and Talbot, 1986). These glands are found in both males and females and must therefore have roles beyond egg attachment. They resemble other tegumental glands structurally and synthesize at least two kinds of secretory granules. These granules are released from females at spawning, but some may be released at other times as well. The glands are complex and will require further work to completely define their functions. Our data indicate however that they do not

form a coat around the egg as others had reported.

Functional Morphology of the Male Reproductive Tract

During the 4-year award period, we acquired considerable expertise with the vas deferens of commercially important crustaceans. We examined the functional morphology of the vas deferens in *Homarus* (Kooda-Cisco and Talbot, 1986), *Cherax* (Talbot and Beach, 1989), and *Penaeus* (Ro et al. in review). Our work with *Penaeus*, the shrimp, has been our prime focus, because the shrimp's tract degenerates rapidly in captivity. This has presented problems for shrimp aquaculturists and precluded closing the life cycle in captivity. The shrimp also has the most complex vas deferens of the three species studied, and the storage ampoule had not previously been analyzed by any laboratory. In all three species, the vas deferens plays a major role in forming the acellular coats of the spermatophore. In *Penaeus*, most of the spermatophore is elaborated in the terminal ampoule. The secretory epithelial lining of the vas deferens releases products by both exocytosis and apocrine secretion. We also analyzed the structure of the normal mature spermatophore. This past summer, we dissected the spermatophore into 11 separate regions and solubilized each region in sample buffer for analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis. We are completing the latter experiments this fall quarter. These observations have been necessary to plan experiments and develop assays to investigate the factors leading to degeneration of the vas in captivity.

Morphological Changes Occurring in the Male Shrimp Reproductive Tract in Captivity

In collaboration with Dr. Lawrence in Texas, we sampled captive males periodically and performed a number of observations on their reproductive tracts. Our observations define, for the first time, a series of changes that occur in the vas deferens, testes, and spermatophore at various times after capture. All males in the study became infertile by about 1 month of captivity even though this was well before any external evidence of a problem. These observations helped us understand the nature of the problem and provided us with sufficient data to conduct further analyses on the causes of this disease. Our observations have been presented at the World Aquaculture meeting in Hawaii and were recently published (Talbot, et al. 1989).

Cooperating Organizations

Aquaculture Enterprises
New England Lobster, Sunset Beach, California
Texas A&M University
University of Paris

References

Goudeau, M., P. Talbot, and R. Harper. 1987. Mechanism of egg attachment stalk formation in the lobster, *Homarus*. *Gamete Res.* 18:279–289.

Harper, R., and P. Talbot. 1984. Analysis of the epibiotic bacteria of lobster (*Homarus*) eggs and their influence on the loss of eggs from the pleopods. *Aquaculture* 36:9–26.

Ishida, T., P. Talbot, and M. Kooda-Cisco. 1986. Technique for the long-term storage of lobster (*Homarus*) spermatophores. *Gamete Res.* 14:183–195.

Johnson, B. and P. Talbot. 1986. Ultrastructural analysis of the pleopod tegumental glands in male and female lobsters, *Homarus americanus*. *J. Crustacean Biol.* 7:288–301.

Kooda-Cisco, M. and P. Talbot. 1986. Ultrastructure and role of the lobster vas deferens in spermatophore formation: The proximal segment. *J. Morphol.* 188:91–103.

Talbot, P., and D. Beach. 1989. In press. Functional morphology of the vas deferens in *Cherax*. *J. Crustacean Biol.*

Talbot, P., and M. Goudeau. 1988. A complex cortical reaction leads to formation of the fertilization envelope in the lobster (*Homarus*). *Gamete Res.* 191:1–18.

Talbot, P., and R. Harper. 1984. Abnormal egg stalk morphology is correlated with clutch attrition in laboratory-maintained lobsters (*Homarus*). *Biol. Bull.* 166:349–356.

Talbot, P., D. Howard, J. Leung-Trujillo, T. W. Lee, W.-Y. Li, H. Ro, and A. L. Lawrence. 1989. Characterization of male reproductive tract degenerative syndrome in captive penaeid shrimp (*Penaeus setiferus*). *Aquaculture* 78: 365–377.

Talbot, P. In review. Structure and function of the vas deferens in the shrimp, *Penaeus setiferus*: Segments 1–3.

Talbot, P., C. Thaler, and P. Wilson. 1984. Spawning, egg attachment and egg retention in captive lobsters (*Homarus americanus*). *Aquaculture* 37:239–249.

Talbot, P., C. Thaler, and P. Wilson. 1986. Artificial insemination of the American lobster (*Homarus*). Gamete Res. 14:25–31.

Publications

Beach, D. and P. Talbot. 1986. Ultrastructural comparison of sperm from the crayfish *Cherax tenuimanus* and *Cherax albidus*. *J. Crustacean Biol.* 7:205–218.

Goudeau, M., P. Talbot, and R. Harper. 1987. Mechanism of egg attachment stalk formation in the lobster, *Homarus*. *Gamete Res.* 18:279–289.

Harper, R., and P. Talbot. 1984. Analysis of the epibiotic bacteria of lobster (*Homarus*) eggs and their influence on the loss of eggs from the pleopods. *Aquaculture* 36:9–26.

Howard, D., L. Haimo, and P. Talbot. 1985. A demonstration of actin and microtubules in non-striated muscle cells of the lobster ovary. *J. Cell Biol.* 101:166a. Abstract.

Ishida, T., P. Talbot, and M. Kooda-Cisco. 1986. Technique for the long-term storage of lobster (*Homarus*) spermatophores. *Gamete Res.* 14:183–195.

Johnson, B., and P. Talbot. 1986. Ultrastructural analysis of the pleopod tegumental glands in male and female lobster, *Homarus americanus*. *J. Crustacean Biol.* 7:288–301.

Kooda-Cisco, M., and P. Talbot. 1984. Formation of the spermatophore wall in the lobster, *Homarus*. *J. Cell Biol.* 4:394a. Abstract.

Kooda-Cisco, M., and P. Talbot. 1986. Ultrastructure and role of the lobster vas deferens in spermatophore formation: The proximal segment. *J. Morphol.* 188:91–103.

Ro, S., P. Talbot, J. Leung-Trujillo, and A. L. Lawrence. 1989. Structure and function of the vas deferens in the

shrimp, *Penaeus setiferus*: Segments 1-3. In review.

Talbot, P. 1984. Problems and progress in controlling reproductive biology in the American lobster (*Homarus*). In *Advances in Invertebrate Reproduction*. W. Engels, ed. Elsevier Science Publishers, New York, pp. 473–480.

Talbot, P. 1989. In press. Ovulation, attachment, and retention of lobster eggs. Invited review.

Talbot, P. 1989. In press. Reproduction in lobsters. In: Reproduction in Marine Invertebrates. C. Hand, and W. Clark, eds. Academic Press.

Talbot, P., and D. Beach. 1989. Functional morphology of the vas deferens in *Cherax*. *J. Crustacean Biol.* 9:9–24.

Talbot, P., and M. Goudeau. 1985. Formation of the fertilization envelope in the lobster, *Homarus*. *J. Cell Biol.* 101:378a. Abstract.

Talbot, P., and M. Goudeau. 1988. A complex cortical reaction leads to formation of the fertilization envelope in the lobster (*Homarus*). *Gamete Res.* 191:1–18.

Talbot, P., and R. Harper. 1984. Abnormal egg stalk morphology is correlated with clutch attrition in laboratory-maintained lobsters (*Homarus*). Biol. Bull. 166:349–356.

Talbot, P., D. Howard, J. Leung-Trujillo, T. W. Lee, W.-Y. Li, H. Ro, and A. L. Lawrence. 1989. Characterization of male reproductive tract degenerative syndrome in captive penaeid shrimp (*Penaeus setiferus*). *Aquaculture* 78:365–377.

Talbot, P., C. Thaler, and P. Wilson. 1984. Spawning, egg attachment and egg retention in captive lobsters (*Homarus americanus*). *Aquaculture* 37:239-249.

Talbot, P., C. Thaler and P. Wilson. 1986. Artificial insemination of the American lobster (*Homarus*). *Gamete Res.* 14:25–31.

Theses

Beach, D. 1986. Ultrastructural comparison of sperm from the crayfish *Cherax tenuimanus* and *Cherax albidus*. Master's thesis, Dept. of Biology, University of California, Riverside.

Johnson, B. 1988. Pleopod tegumental glands in the American lobster. Master's thesis, Dept. of Biology, University of California, Riverside.

Dissertations

Howard, D. will finish 1990.

Lectures and Presentations at Conferences

Gerrero, R. and P. Talbot. A microscopic study of the setae on the necrotic pleopods of the American lobster. Presented at West Coast Regional Developmental Biology Conference, Asilomar, California, 1984. Abstract.

Goudeau, M., P. Talbot, and R. Harper. Mechanism of egg attachment stalk formation in the lobster, *Homarus*. Presented at the meeting of the American Society of Zoologists, New Orleans, Louisiana, December, 1987.

Goudeau, M., P. Talbot, and R. Harper. Mechanism of egg attachment in the lobster *Homarus*. Presented at the meeting of the World Aquaculture Society, Honolulu, Hawaii, January, 1987.

Howard, D., L. Haimo, and P. Talbot. Microtubules in non-striated muscle cells. Presented at the West Coast Regional Developmental Biology Conference, Asilomar, California, 1985.

Howard, D. and P. Talbot. Role of Ca^{2+} and cAMP in contraction of microtubule-containing muscles. Presented at the 4th International Congress Cell Biology, Montreal, Canada, August, 1988.

Howard, D., and P. Talbot. Neurohormones involved in spawning in the lobster *Homarus americanus*. Accepted for presentation at the World Aquaculture Society meetings, Los Angeles, Califonria, 1989.

Ishida-Schick, T., P. Talbot, and M. Kooda-Cisco. A procedure for the long term storage of lobster (*Homarus*) sperm. Presented at the annual meeting of the World Mariculture Society, 1984.

Johnson, B., and P. Talbot. Comparison between pleopod tegumental glands in male and female American lobsters. Presented at West Coast Regional Developmental Biology Conference, Asilomar, California, 1984.

Johnson, B. and P. Talbot. Tegumental glands in the pleopods of the female American lobster (*Homarus americanus*). Presented at the annual meeting of the World Mariculture Society, Vancouver, British Columbia, 1984.

Kooda-Cisco, M., and P. Talbot. The lobster vas deferens: Ultrastructure and role in spermatophore formation. Presented at 16th annual meeting of the World Mariculture Society, Orlando, Florida, 1985.

Ro, S. and P. Talbot. Structure and function of the vas deferens in *Penaeus setiferus*. Presented at the 4th International Congress Cell Biology, Montreal, Canada, August, 1988.

Ro, S., P. Talbot, J. Trujillo, and A. Lawrence. Structure and function of the male reproductive tract in *Penaeus setiferus*. Presented at the World Aquaculture Society Meetings, Honolulu, Hawaii, January, 1987.

Talbot, P. Lobster eggs. Departmental seminar, Bodega Marine Laboratory, Bodega Bay, California, Spring. 1986.

Talbot, P. Ovulation, spawning and egg attachment in the lobster, *Homarus americanus*. Invited symposium talk, American Society of Zoologists, New Orleans, California, December, 1987.

Talbot, P., and D. Beach. The role of the vas deferens in spermatophore formation in the crayfish *Cherax*. Accepted for presentation at the World Aquaculture Society meeting, Los Angeles, 1989.

Talbot, P., and M. Goudeau. Formation of the fertilization envelope in the lobster, *Homarus*. Presented at the annual meeting of the World Aquaculture Society, Reno, Nevada, January, 1986.

Talbot, P., D. Howard, J. Trujillo, T. Lee, L. Li, H. Ro, and A. Lawrence. Unexpected rapid destruction of sperm in captive penaeid shrimp. Presented at the meeting of the World Aquaculture Society, Honolulu, Hawaii, January, 1987.

Talbot, P., C. Thaler, and P. Wilson. Artificial insemination of lobsters (*Homarus*). Presented at the 16th annual meeting of the World Mariculture Society, Orlando, Florida, 1985.

Culture of Marine Bivalves: Utilization of Dissolved Amino Acids

University of California, Irvine
R/A-60
Project Initiated: October 1, 1986
Project Completed: September 30, 1987

Grover C. Stephens

I will deal mainly with the accomplishments of this program during the period October 1, 1986, through September 30, 1987. In doing this, I will briefly summarize progress since the inception of this project on September 30, 1981. Details and references can be found in previous annual reports.

At the time we began this work, we and others, by using radiolabeled substrate, had demonstrated rapid entry of free amino acids (FAA) from seawater into bivalves of economic interest. At that time, no technique was available for direct chemical demonstration of net entry of FAA from the very dilute solutions that characterize the inshore marine environment. Therefore, there were reasonable reservations about the reality of the phenomenon based on the possibility of leakage or exchange diffusion of FAA from the very concentrated intracellular pools of these animals.

We applied the newly introduced technique of determination of FAA in seawater by high-performance liquid chromatography (HPLC) to this field for the first time. This made it possible to demonstrate clearly the net entry of FAA both from artificial solutions mimicking the natural abundance of environmental FAA and from natural inshore marine waters in the immediate habitat of the bivalves studied. Furthermore, we compared entry rates of labeled substrates with net entry as measured by HPLC and showed that no leakage or exchange diffusion of amino acids occurred.

At this time, another issue in the literature was the effect of contaminant bacteria and other microorganisms on the rates of uptake of FAA observed in laboratory experiments. A major school of thought contended that marine invertebrates such as bivalves could not compete effectively with marine bacteria for available FAA. We developed axenic (bacteria-free) preparations of echinoderm larvae as model systems to explore this issue. Using this material, we have shown that adherent microorganisms play no role in uptake of FAA observed in laboratory experiments. We also showed that larvae are indeed capable of effective competition with microorganisms in the water column for dissolved resources naturally available. We proceeded to show by indirect methods that this also is true for bivalves of economic interest.

In the period from October 1, 1986 through September 30, 1987, we devoted our efforts to another issue. Investigators studying uptake of FAA by the mussel *Mytilus* in the United States and abroad argued that although FAA were taken up into the epidermis of this animal, they were not translocated to deeper tissues. This implied that the process could not contribute to the general nutrition of the animals. Rather, they interpreted uptake as serving the following two purposes: nutrition of the superficial epidermis itself and compensation for intercellular loss of substrate across the epidermis by recapture of lost substrate.

In a series of papers, Rice and Stephens showed that translocation of FAA acquired by epidermal uptake to deeper tissues occurred rapidly and effectively. Animals used in the studies were the major bivalves of economic interest: mussels, oysters, and clams. This work formed the substance of the Doctoral dissertation of Michael A. Rice.

A major invited review of the field appeared in *Biochimica Biophysica Acta* in 1988. This reviews the work discussed here along with the general field of epidermal transport of free amino acids in marine organisms.

Publications

Davis, J. P., S. Bellis, and G. C. Stephens. 1988. Characterization of a broad-scope amino acid transport system in larval and adult sand dollars. *Am. J. Physiol.* 254:R485-R490.

Rice, M. A. 1987. Transepithelial flux of amino acids in three species of marine bivalve mollusks. Doctoral dissertation, University of California, Irvine.

Rice, M. A., and G. C. Stephens. 1988. Influx, net flux and transepithelial flux of amino acids in the hardshell clam *Mercenaria mercenaria* (Linne): Influence of salinity. *Comp. Biochem. Physiol.* 89A(4):631–636.

Rice, M. A., and G. C. Stephens. 1988. Influx and transepithelial flux of amino acids in the mussel, *Mytilus edulis*. *J. Exp. Biol.* 135:275–287.

Rice, M. A., and G. C. Stephens. 1987. Uptake and internal distribution of exogenously supplied amino acids in the Pacific oyster, *Crassostrea gigas* (Thunberg). *Aquaculture.* 66:19–31.

Stephens, G. C. 1988. Epidermal amino acid transport in marine invertebrates. *Biochim. Biophys. Acta.* 947:113–138.

Reproduction and Growth in Crustacean Aquaculture

University of California, Davis
Bodega Marine Laboratory
R/A-61
Project Initiated: October 1, 1984
Project Completed: September 30, 1987

Ernest S. Chang, Wallis H. Clark, and Douglas E. Conklin

Endocrine Regulation of Crustacean Growth and Reproduction (E. S. Chang)

In order to grow, a crustacean must first replace its confining, rigid exoskeleton with a larger one and subsequently fill it with tissue. This periodic shedding of the old exoskeleton is accomplished by molting, which is the external manifestation of a discontinuous growth process. This molting process is under the direct control of the molting hormone, 20-hydroxyecdysone (Figure 1). The actual control of the synthesis and secretion of the precursor of the molting hormone (ecdysone, Figure 1) is mediated by the peptide molt-inhibiting hormone (MIH) (see Chang, 1985, for a review). A major thrust of our research has been the isolation and characterization of MIH.

Recent research by our laboratory (Chang et al., 1987) has shown that MIH can be isolated as a single peptide by high-performance liquid chromatography (HPLC). We have obtained an amino acid composition of the lobster MIH. From these data, a molecular weight of 8700 kd was obtained. These studies now permit us to complete this phase of the project by collecting sufficient amounts of MIH for amino acid sequencing studies.

Recently, we have determined that the maximal effect of injections of MIH occurs 48 hr after injection (Figure 2).

In related studies with various collaborators, we have determined that peak VIII from the tropical crab (*Cardisoma carnifex*) does not possess any MIH activity in our lobster-assay system. In addition, compounds that have MIH activity in the blue crab (*Callinectes sapidus*) or the Japanese swimming crab (*Portunus trituberculatus*) do not possess MIH activity in the lobster.

The role of the eyestalk in the control of larval development and metamorphosis has also been investigated. It was observed that eyestalk ablation of second-stage lobster larvae resulted in an accelerated rise in the titers of whole larval ecdysteroid (molting hormone) during the third stage. The appearance of both the premolt ecdysteroid peak and the subsequent ecdysis were accelerated by eyestalk removal during the previous molt interval. Replacement therapy using extracts of sinus glands from juvenile lobsters injected into third-stage larvae (ablated during the second stage), delayed the next molt. This delay was significantly longer than for larvae injected with extracts of non–sinus gland eyestalk tissue.

Extracts of sinus glands similarly decreased ecdysteroid titers of ablated larvae within 12 hours. Basal levels were maintained in larvae injected with extracts of sinus glands; control larvae reached the premolt peak. These results indicate that a molt-inhibitory mechanism similar to that of juvenile and adult decapod crustaceans may also exist in larvae.

Several decades ago, it was postulated that an androgenic hormone is present in male crustaceans that influences male primary and secondary sexual characteristics. More recently, the chemical structure of the putative androgenic hormone has been reported (Ferezou et al., 1977). However, this material has not been used *in vivo* to determine if it is able to transform juvenile female crustaceans into males. Using sixth-stage sibling lobsters, we were able to determine their sex from external morphological characteristics. We then initiated a series of injections into these females of either farnesyl acetone or control substances. After several molts, we did not observe any significant differences between the experimental and the control groups, thus indicating that farnesyl acetone may not be functioning as the androgenic hormone in lobsters.

This last year we have completed a small project related to the eyestalk mediation of crustacean tissue hydration that directly affects

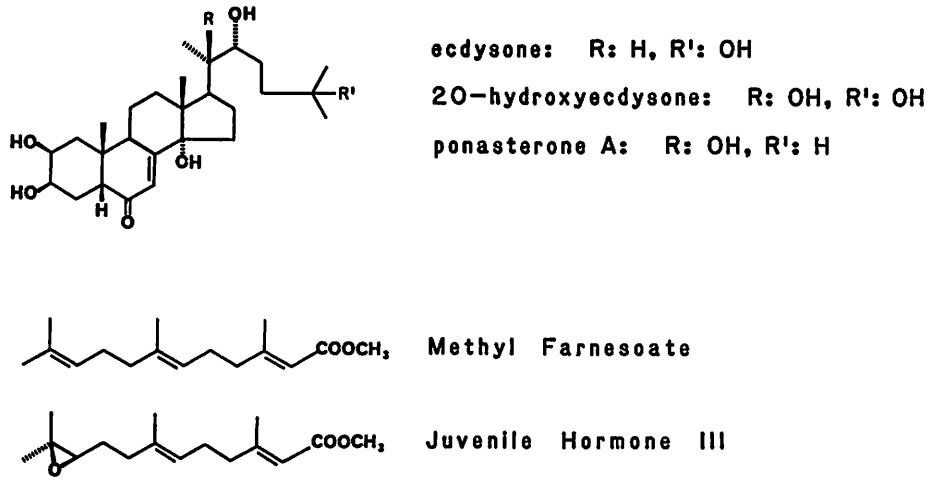


Figure 1. Structures of some of the compounds discussed in the text.

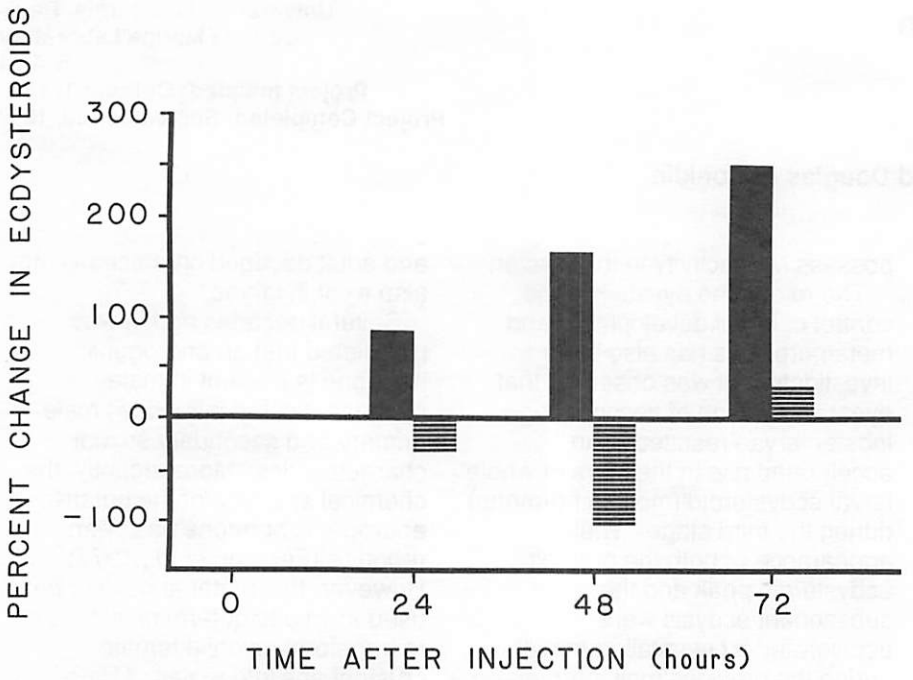


Figure 2. Effects of injections of extracts from sinus glands on circulating ecdysteroids in lobsters. The animals were 7 months old, and their eyestalks were ablated 48 hr after they had molted. Six days later, 10 µl of hemolymph were taken for radioimmunoassay of ecdysteroids. Immediately afterwards, an acid extract of two sinus glands was injected (hatched bars). Control lobsters were eyestalk-ablated siblings that were injected with an equivalent mass of non–sinus gland eyestalk neural tissue (solid bars). They were then assayed for circulating ecdysteroids at 24, 48, and 72 hr after injection. Separate groups of animals were used for each time point. The data are presented as the percentage of change from the level at the time of injection (0 hr). The data are significant at $P < .001$, $P < .05$, and $P < .01$ for the 24-, 48-, and 72-hr time points, respectively. Number of lobsters used ranged from 5 to 15. (Chang and Bruce, unpublished observations.)

Table 1. Water Measurements on Control and Eyestalk-Ablated (EA) Lobsters (n = 5 for each measurement)

Tissue Source	Tissue Water (g H_2O/g dry tissue ± S.D.)	Extracellular Space (% of wet tissue ± S.D.)	Cellular Water (g H_2O/g dry cells ± S.D.)
Hepatopancreas			
Control	2.278 ± 0.285	18.3 ± 0.2	1.767 ± 0.188
EA	3.567 ± 0.474	19.6 ± 0.3	2.763 ± 0.550
	$P < .01$	$P < .001$	$P < .01$
Abdominal Muscle			
Control	3.404 ± 0.135	11.8 ± 0.3	2.970 ± 0.154
EA	4.382 ± 0.230	23.5 ± 0.4	3.298 ± 0.200
	$P < .01$	$P < .0001$	$P < .05$

wet-weight gains during growth. Inasmuch as crustaceans must take up large amounts of water after molting to expand their soft, new exoskeleton, the study of this process is relevant to the goals of this subproject.

Although several studies in this general area have been conducted by others, they have concentrated on alterations in the osmolarity or ionic composition of the hemolymph or on the permeability of the gills, gut, and integument (Mantel and Farmer, 1983). In contrast, little is known about the effects of eyestalk ablation on tissue and cellular hydration. The purpose of this study was to determine the effects of eyestalk removal upon several aspects of water balance in the American lobster.

Both extracellular and intracellular water contents were significantly higher in abdominal muscle and hepatopancreas from eyestalk-ablated lobsters compared with controls (Table 1). The density and dry solids content of hemolymph from ablated lobsters were less than controls (Table 2), although hemolymph osmotic pressures of both groups were indistinguishable. These findings have important implications about the macromolecular and ionic composition of hemolymph. In addition, the data indicate that there may be a diuresis-regulating hormone in the crustacean eyestalk. These studies will be pursued.

In insects and crustaceans, molting is mediated by 20-hydroxyecdysone. Insects have another chemical factor that regulates development, juvenile hormone (JH, Figure 1) (for review, see Downer and Laufer, 1983). Juvenile hormone, though isolated many years ago in insects, has not been isolated from crustacean sources. There are indications that JH may be present in crustaceans (Schneiderman and Gilbert, 1958). After collecting and extracting large quantities of lobster tissues, we extracted them for JH and, in collaboration with Zoecon Research Institute, concluded that JH is not present in lobsters. However, its

precursor, methyl farnesoate (MF, Figure 1), is present as determined by gas-liquid chromatography and mass spectroscopy.

When added to the water in which lobster larvae were cultured, methyl farnesoate did result in a lengthened larval life (times to metamorphosis of 20.8 ± 1.5 and 22.0 ± 1.3 days for the control and MF-treated larvae, respectively; $P < .01$). This result is consistent with the action of MF acting as a juvenile-promoting factor.

A final aspect of this subproject has been the development of an established cell line from crustacean tissues. We currently have cultures that routinely grow and divide for more than 8 months *in vitro*. These cultures, from lobsters and crayfish, are responsive to the crustacean molting hormone, 20-hydroxyecdysone (Figure 3). This should be a valuable model system for studying how the hormone mediates effects at the cellular level that result in molting and increased growth.

Table 2. Hemolymph Variables in Control and Eyestalk-Ablated Lobsters (n = 5 for each measurement)

	Density (g/ml ± S.D.)	Dry Solids (mg/µl ± S.D.)	Osmotic Pressure (mosmol/kg ± S.D.)
Control	1.022 ± 0.006	0.064 ± 0.019	926 ± 13
Eyestalk-Ablated	1.012 ± 0.005	0.031 ± 0.010	925 ± 10
	$P < .05$	$P < .05$	Not significant

Reproductive Biology of Shrimp
(W. H. Clark)

The events of gamete activation and fusion have been well documented in several vertebrate and invertebrate species (Gwatkin, 1977; Epel and Vacquier, 1978; Lopo, 1983). In the majority of the systems studied, flagellated sperm are involved. As these cells approach an egg, they encounter one or more investment coats, interact with one of these coats, and undergo acrosomal activation. As the acrosome is activated, it undergoes exocytosis, a prerequisite for fertilization. This exocytosis externalizes binding protein for sperm–egg adhesion and intercellular membranes required for sperm–egg fusion.

Recent studies have shown that controls to ensure species specificity of sperm–egg interaction are present. Examples of this specificity are acrosomal activation (SeGall and Lennarz, 1979; Aketa and Ohta, l979; Cherr and Clark, 1983) and binding of acrosome-reacted sperm to eggs (Summers and Hylander, 1976; Glabe and Lennarz, 1979; Schmell and Gulyas, 1980).

Increased understanding of gamete interactions and the control of these interactions provide the basis for successful gamete manipulations important to both basic and applied research. Unfortunately, many species for which control is desirable possess atypical nonflagellated gametes about which little information is available. This is particularly true in the decapod crustaceans, in which great diversity exists, and most of the information concerning any one species is fragmentary. The only exception to this is our understanding of sperm–egg interaction in the penaeid, *Sicyonia ingentis* (Clark et al., 1984).

The sperm of *S. ingentis* demonstrate several events prerequisite to fertilization: (1) Sperm must capacitate in the female thelycum before being competent. (2) Sperm bind with the vitelline envelope before acrosomal activation. (3) Sperm undergo spike retraction and acrosomal exocytosis in response to an egg investment component. (4) Sperm secondarily bind to the egg surface. (5) In response to an egg jelly component, sperm form an acrosomal filament.

We have shown that the primary and secondary events of acrosomal activation in the sperm of *S. ingentis* are initiated by separate inducers and that the inducer of the secondary event (filament formation) is an inhibitor-sensitive protein (Griffin et al., 1985) (Figure 4).

Attempts to further understand gamete interaction in our penaeid model system, *S. ingentis*, have continued. Efforts have concentrated on the secondary

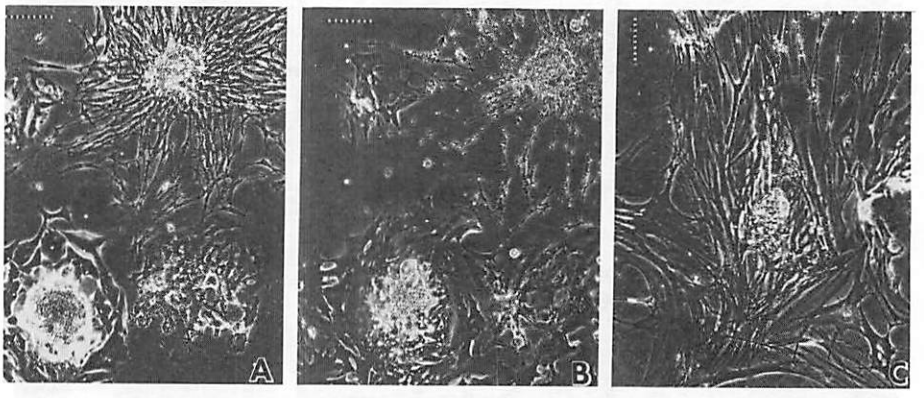


Figure 3. Primary culture of lobster testes after 2 months without any addition of hormone (A). 20-Hydroxyecdysone (10^{-8} M) was then added. Six days after addition of hormone (B), the cells in the upper colony have started to die, whereas the cells in the lower colony are proliferating. The hormone was then withdrawn. The upper colony has disappeared, and the cells of the lower colony have continued their proliferation 21 days after withdrawal of hormone (C). Scale bars = 200 µm. (Brody and Chang, unpublished observations.)

event of acrosomal activation (acrosomal filament formation) and have resulted in two exciting discoveries: (1) As a part of studies into the ionic requirements of acrosomal activation in *S. ingentis* sperm, we have measured changes in intracellular pH. These measurements show that, unlike other sperm and cells in general, *S. ingentis* sperm undergo a decrease in intracellular pH during activation and that the drop in pH is associated with filament formation (Griffin et al., 1987). (2) Furthermore, we have shown that the activity of a trypsinlike enzyme of egg origin is necessary for the formation of jelly around the egg and appears to be the inducer of filament formation in the sperm.

On the basis of findings in one species, *Penaeus californiensis*, we reported that the sperm of closed thelyca *Penaeus* species appear to undergo two binding events but only one activational event (acrosomal exocytosis). The same events, primary binding, acrosomal exocytosis, and secondary binding, have been observed in another closed thelycum *Penaeus, Penaeus aztecus*; we have not seen the formation of an acrosomal filament in sperm of closed thelyca *Penaeus* species. When incubated with conspecific egg-derived inducers, *P. aztecus* sperm that have been removed from female seminal receptacles undergo acrosomal exocytosis. In contrast, sperm removed from males do not respond to conspecific egg-derived inducers, indicating that a capacitation process is operational in closed thelyca *Penaeus* species as well as the closed thelyca *Sicyonia* species.

Studies were started to document the activational changes that occur in the sperm of the open thelycum penaeid, *Penaeus setiferus*. We have examined activation during sperm–egg interaction *in vivo* and during *in vitro* activation by using isolated sperm and inducers collected from spawned *P. setiferus* ova. *P. setiferus* sperm undergo activational changes that in no way resemble those of closed thelyca penaeids (neither *Sicyonia* species nor *Penaeus* species). The documentation of these activational changes was undertaken through a collaborative ultrastructural study with Dr. William Dougherty. Dr. Dougherty has been studying the ultrastructure and histochemistry of the spermatophore in *P. setiferus*. We have determined that sperm removed from males as well as sperm taken from spermatophores subsequent to mating undergo these changes in response to egg-derived inducers. These results indicate that a capacitation process is not operational in the open thelycum penaeids. Verification of this, however, must await examination of other species of open thelycum penaeids.

Studies to determine the extent of species specificity at the gamete level within the genus *Sicyonia* have yielded the following: (1) *Sicyonia brevirostris* sperm undergo primary binding to *S. ingentis* eggs and exhibit acrosomal exocytosis. (2) After exocytosis, *S. brevirostris* sperm do not form acrosomal filaments. These studies have been repeated, and the reciprocal experiments have been conducted. Sperm of both *S. ingentis* and *S. brevirostris* undergo acrosomal exocytosis in the presence of the contraspecific egg-derived inducer, but neither form acrosomal filaments. This suggests that specificity between these two species lies in the ability to induce an acrosomal filament. In light of discoveries about the changes in intracellular pH and the enzymatic induction of filament formation in *S. ingentis* sperm, it would be of interest to determine if the same changes occur in *S. brevirostris* sperm and if one or both of these are the control points of specificity.

As we continue to unravel the events of gamete interaction and activation in other penaeids, the importance of *S. ingentis* as a model system becomes more evident. For example, techniques devised with *S. ingentis* for the collection of egg-derived inducers of the acrosome reaction using light-cycled females (Figure 5) have now been directly applied to inducer collection in *S. brevirostris, P. aztecus* and *P. setiferus*. Additionally, our understanding of the changes and their controls during the activation of sperm in *S. ingentis* is being used as a basis from which we can analyze similar or dissimilar events in the sperm of the other penaeids.

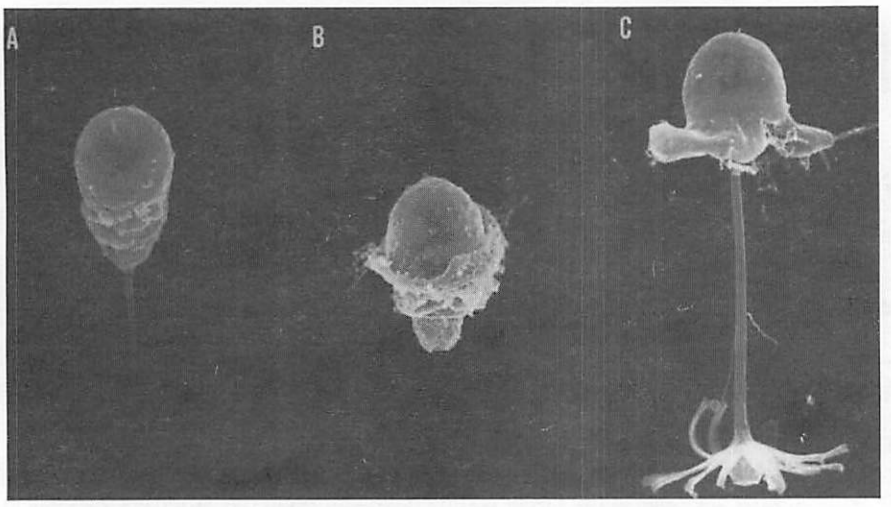


Figure 4. Scanning electron micrographs of (A) an unreacted *S. ingentis* sperm, (B) a sperm that has undergone acrosomal exocytosis, and (C) a sperm that has completed the acrosome reaction and possesses an acrosomal filament (x 10,000).

Role of Ascorbic Acid in Crustacean Growth and Reproduction (D. E. Conklin)

Classically, it had been assumed that all fish and invertebrate species

required a dietary source of vitamin C (L-ascorbic acid). Dietary deletion experiments with many species of fish (see reviews by Lovell, 1984; Sandnes, 1984) and some crustaceans (Guary et al., 1976; Lightner et al., 1979) have shown that lack of vitamin C has a negative effect on growth, health, and reproductive success. In that ascorbic acid is also both very labile chemically and highly water-soluble, provision of appropriate dietary levels of vitamin C is an important issue for aquatic animal nutritionists. The amount of dietary ascorbic acid required by any animal is influenced by two primary factors: ascorbate biosynthetic ability, if any, and physiological demand. A third component, tissue storage, can substitute for dietary input, at least on a temporary basis. Our goal has been to define these factors for crustaceans of aquacultural importance to develop superior formulated diets, particularly for reproduction.

In animals that can synthesize it, ascorbic acid is produced from glucose through a short pathway leading to L-gulonolactone (Burns, 1957, 1967; Sato et al., 1976). The enzyme L-gulonolactone oxidase (GLO) converts this lactone to 2-keto-L-gulonate, which spontaneously isomerizes into L-ascorbic acid. For species that have been examined, the lack of synthesis is due to the absence of GLO (Sato and Udenfriend, 1978). Using GLO activity as a guide, Chatterjee (1973) proposed a scheme explaining the evolution of ascorbic acid biosynthesis, which excluded all fish and invertebrates. On the basis of surveys of about 50 species, ranging from insects to mammals, Chatterjee concluded that ascorbic acid biosynthesis originated in the amphibian kidney. It has consequently been assumed that all fish and crustaceans, as well as other invertebrates, would be incapable of synthesis and would have an absolute requirement for dietary vitamin C. Although this evolutionary scheme advanced by Chatterjee has been widely accepted, it is *erroneous*.

Research by our laboratory indicates that ascorbic acid biosynthesis arose much earlier than amphibians. GLO activity has now been found in all classes of fish (Conklin et al., in prep.) as well as some invertebrates. We have been able to confirm the earlier report (Wallace et al., 1985) of GLO activity in the horseshoe crab and also found it in an echinoderm (unpublished data). Establishment of this pathway in various taxa of both deuterostome and protostome lineages suggests an origin at least concurrent with early metazoans. Work by Nishikimi and coworkers (1978) with yeast suggests an even earlier origin with the first heterotrophic organisms. These findings provide a completely new perspective on the evolution of ascorbic acid biosynthesis.

Surprisingly, even though ascorbate biosynthesis has been maintained throughout the animal kingdom, numerous species have a dietary requirement for vitamin C and/or lack GLO. In related work on fish, which started with dietary studies on the white sturgeon (Sea Grant Project R/A-90), loss of GLO activity was shown not to follow any clear phylogenetic pattern. High levels of GLO activity were found for all of the earlier evolved cartilaginous fish species examined (see Table 3). However, among the later derived teleosts, the majority of species that have been tested (some 30 species) can be considered non-synthesizers on the basis of GLO activity (Chatterjee, 1973; Conklin et al., in prep.; Soliman et al., 1985; Wilson, 1973) and/or dietary deletion studies (see Milliken, 1982 for review). Although most teleosts are unable to synthesize ascorbic acid, there are some noteworthy exceptions.

Overall, the pattern of GLO activity in fish seems to be quite spotty, with no real correlation with either phylogeny or biological specialization. In studies involving enzyme activity (Yamamoto et al., 1978), radioisotopic labeling (Ikeda and Sato, 1964), and dietary deletion (Sato et al., 1978), the common carp *Cyprinus carpio* was shown to synthesize ascorbic acid, utilizing GLO at rates sufficient to meet normal physiological demands over a 56-week period. Yamamoto

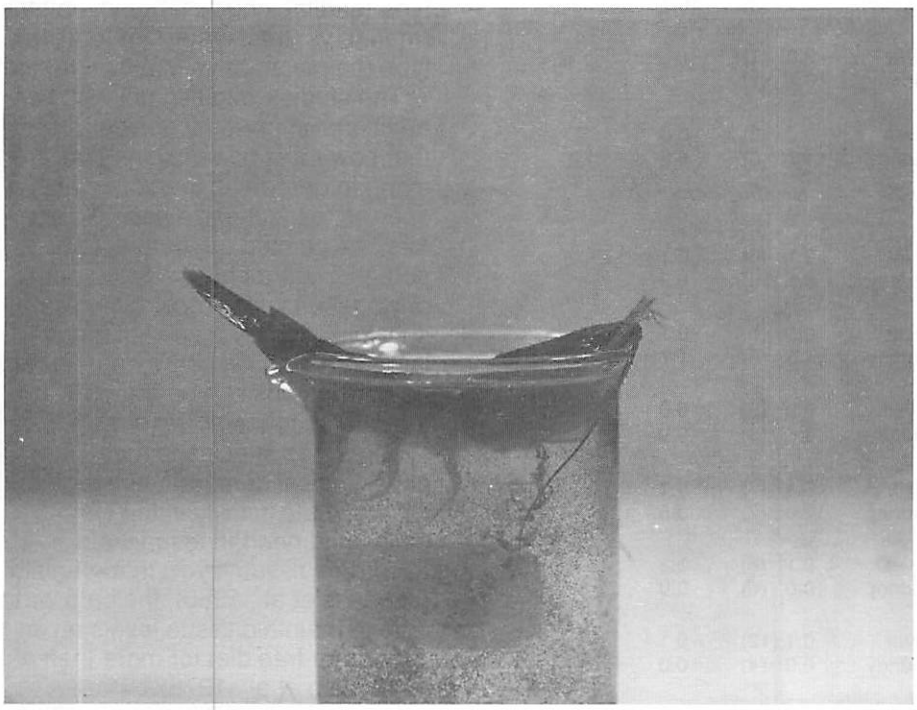


Figure 5. An *S. ingentis* female spawning on a beaker containing seawater.

Table 3. L-Gulonolactone (L-GL) Oxidase Activity in Fish

Species	Synthesis	Tissue	L-GL (μg/g/hr) Mean (n)	S.D.
Class: Myxini				
Pacific hagfish				
Eptatretus stouti	+	Liver	4.4 (12)	2.1
	+	Kidney	3.2 (4)	1.0
Class: Cephalaspidomorphi				
Sea lamprey				
Petromyzon marinus	–	Liver	0.1 (9)	0.1
	+	Kidney	2.4 (9)	2.4
Pacific lamprey				
Lampetra tridentata	+	Liver	2.0 (4)	1.8
	+	Kidney	51.0 (3)	20.2
Class: Chondrichthyes				
Spotted ratfish				
Hydrolagus colliei	–	Liver	0.1 (2)	
	+	Kidney	0.5 (2)	
Sevengill shark				
Notorynchus cepedianus	+	Kidney	1.3 (6)	0.4
Brown smoothhound				
Mustelus henlei	–	Liver	0.3 (3)	0.4
	+	Kidney	38.1 (6)	37.4
Leopard shark				
Triakis semifasciata	–	Liver	0.2 (3)	0.2
	+	Kidney	26.6 (18)	7.2
Soupfin shark				
Galeorhinus galeus	+	Liver	11.9 (1)	
	+	Kidney	15.6 (3)	1.7
Spiny dogfish				
Squalus acanthias	+	Kidney	5.4 (3)	1.2
Atlantic stingray				
Dasyatis sabina	–	Liver	0.1 (3)	0.1
	+	Kidney	2.4 (3)	0.7
Pacific electric ray				
Torpedo californica	–	Liver	0.0 (1)	
	+	Kidney	10.4 (1)	
Big skate				
Raja binoculata	–	Liver	0.0 (3)	0.0
	+	Kidney	11.7 (6)	6.8
Class: Osteichthyes				
White sturgeon				
Acipenser transmontanus	–	Liver	0.1 (6)	0.1
	+	Kidney	6.6 (13)	6.9
Shortnose sturgeon				
Acipenser brevirostrum	–	Liver	0.1 (5)	0.1
	+	Kidney	4.8 (5)	1.7
Florida gar				
Lepisosteus platyrhincus	–	Liver	0.0 (3)	0.0
	+	Kidney	3.6 (3)	0.9
Bowfin				
Amia calva	–	Liver	0.1 (7)	0.1
	+	Kidney	17.0 (7)	3.5
Arowana				
Osteoglossum bicirrosum	–	Liver	0.1 (6)	0.2
	–	Kidney	0.0 (6)	0.0
American eel				
Anguilla rostrata	–	Liver	0.1 (12)	0.1
	–	Kidney	0.0 (4)	0.0
Northern anchovy				
Engraulis mordax	+	Liver	0.5 (1*)	
	–	Kidney	0.1 (1*)	

and coworkers (1978) also found GLO activity in the goldfish *Carassius auratus*, and two other Japanese species, the ugi, *Tribolodon hakonensis*, and a catfish, *Siluris (Parasilurus?) asotus*. In addition to the findings of our own studies, GLO activity in either the carp or goldfish has been confirmed by two other groups (Thomas et al., 1985; Soliman et al., 1985) by independent enzymatic assay techniques as well as by histochemical methods. These latter studies also found GLO activity in the mullet and in some, but not all, species of tilapia. The two tilapia, *Orechromis spilurus* and *Orechromis aureus*, and the mullet, *Mugil cephalus*, are important because of their classification within the order Perciformes, considered to be a phylogenetically advanced fish taxon. Although there does appear, at present, to be a general concentration of synthesizing species associated with the less advanced orders, this may be only a sampling artifact due to our focus on these taxa. Again, with biological specialization there are some trends, but no consistent relationships exist between the ability to synthesize and skeletal type (cartilaginous vs. bony), habitat (marine vs. freshwater), or feeding type (carnivorous vs. omnivorous).

The studies with fish point up two additional important considerations that now must be considered in trying to determine the potential of a species to synthesize ascorbic acid. First, as pointed out before, this potential cannot be predicted from phylogenetic position or biological specialization. Second, although large variations in activity are found, no conclusions can be drawn from the rate as to the ability of a synthesizing species to meet physiological demand. Although the level of GLO activity in the carp is below that needed to maintain ascorbate tissue levels in mammals (Jenness et al., 1980), the carp was able to maintain tissue levels on an ascorbate-free diet for more than a year (Sato et al., 1978).

Discrimination of low but positive values of GLO activity, such as that

Table 3 (continued)

Species	Synthesis	Tissue	L-GL (µg/g/hr) Mean (n)	S.D.
Goldfish				
Carassius auratus	+	Liver	0.4 (8)	0.2
	–	Kidney	0.0 (3)	0.1
Rainbow trout				
Salmo gairdneri	–	Liver	0.0 (10)	0.1
	–	Kidney	0.1 (11)	0.1
Stripped bass				
Morone saxatilis	–	Liver	0.0 (6)	0.0
	–	Kidney	0.0 (2**)	

* Tissue pooled from 12 individuals.

** Two tissue samples, each pooled from three different individuals.

shown by the carp, and variation within the assay test itself have been major problems. Early tests with the horseshoe crab and the lobster, both of which had been reported to synthesize ascorbic acid, were inconclusive because of excessive variation in the assay method that obscured possible low positive results in these two species. Consequently, much of our work this year has focused on refinement of the GLO assay (Ayaz et al., 1976). This assay, which avoids the variable losses of enzyme involved with microsomal isolation, was developed in the laboratory of Dr. Robert Jenness. Some of the work on further refining this assay this year was done in collaboration with Dr. Jenness during a month-long visit to our laboratory.

Subtle but critical differences in technique were found to be necessary for isolating GLO from some of the invertebrates, such as the horseshoe crab, in contrast to that used with vertebrate tissue. Generally, GLO is solubilized by homogenizing tissue in a buffered solution of sodium deoxycholate. The solubilized enzyme is then separated from cell debris by centrifugation. These two steps with the horseshoe crab lead to an apparent loss of activity. Low but clearly positive activity is noted in horseshoe crab tissue when sodium deoxycholate is not used and debris is removed by filtration through glass wool. Other refinements in technique were developed to improve efficiency, as well as a modified HPLC procedure (Bianchi and Rose, 1985) for confirmation purposes. Regardless of the technique used, no GLO activity was found in lobster tissue, and we have concluded that contrary to our original ideas, the lobster is unable to synthesize ascorbic acid. The idea that the American lobster *Homarus americanus* was capable of ascorbic acid biosynthesis was based on some Canadian studies (Desjardins et al., 1985; Kean et al., 1985) as well as dietary studies in our own laboratory. We had hoped to use this species in combination with shrimp, which have been shown to require vitamin C, to investigate the role of ascorbic acid in larval development of crustaceans. This approach would have avoided the lengthy and large-scale dietary trials necessary to alter vitamin C content of the eggs through dietary manipulation of the females. Unfortunately, the basic premise that the two crustaceans differed in biosynthetic ability proved to be false. We have now concluded that the lobster is unable to synthesize the ascorbate needed to meet physiological demands. Early dietary trials, carried out both in Canada and in our laboratory, were of insufficient length to detect the comparatively low requirement of lobsters. We found that juvenile lobsters maintained on a formulated diet (Conklin et al., 1980) without added ascorbic acid were unable to maintain ascorbate tissue levels and eventually died. These results are in agreement with our enzymatic work and although neither enzymatic nor dietary studies can exclude ascorbic acid biosynthesis at a nondetectable level, such a level would be inconsequential to cultured lobsters.

The lack of any discernible pattern related to the presence of ascorbic acid biosynthesis gives added importance to availability of an efficient and effective assay for GLO. We expect to use this assay to further define the ascorbic acid biosynthetic ability of various animals of aquacultural interest. Further dietary studies will be necessary to define the role of ascorbic acid in reproductive success for those species of crustaceans that lack GLO and the ability to synthesize ascorbic acid.

Cooperating Organizations

Aquaculture Production Technology, Ltd., Jerusalem, Israel
Halifax Fisheries Research Laboratory, Canada
Hebrew University of Jerusalem, Israel
Louisiana State University, Baton Rouge and New Orleans
Medical School of Charleston, South Carolina
National Taiwan University, Taipei
Primate Research Institute, Holloman Air Force Base, New Mexico
Stanford University, Stanford, California
Suntory Institute for Bioorganic Research, Osaka, Japan
Texas A&M University, College Station
Tokyo Institute of Technology, Japan
University of California, Los Angeles
University of California, San Diego
University of Connecticut, Storrs
University of Hawaii, Honolulu
University of Illinois, Normal
Zoecon Research Institute, Palo Alto, California

References

Aketa, K., and T. Ohta. 1979. Possible causal relation between the acrosome reaction and cross-fertilization in the sea urchins *Hemicentrotus pulcherrimus* and *Psudocentrotus depressus*. *Gamete Res.* 2:15–23.

Ayaz, K., R. Jenness, and E. C. Birney. 1976. An improved assay for L-gulonolactone oxidase. *Anal. Biochem.* 72:161–171.

Bianchi, J., and R. C. Rose. 1985. The chromatographic separation and radiochemical quantification of ascorbic acid, dehydroascorbic acid and deketogulonic acid. *J. Micronutr. Anal.* 1:3–11.

Burns, J. J. 1957. Missing step in man, monkey and guinea pig required for the biosynthesis of L-ascorbic acid. *Nature* 180:553.

Burns, J. J. 1967. Ascorbic acid. In *Metabolic Pathways,* vol. 1. D. M. Greenberg, ed. Academic Press, New York. pp. 349–411.

Chang, E. S. 1985. Hormonal control of molting in decapod Crustacea. *Am. Zool.* 25:179–185.

Chang, E. S., M. J. Bruce, and R. W. Newcomb. 1987. Purification and amino acid composition of a peptide with molt-inhibiting activity from the lobster, *Homarus americanus. Gen. Comp. Endocrinol.* 65:56–64.

Chatterjee, I. B. 1973. Evolution and the biosynthesis of ascorbic acid. *Science* 182:1271–1272.

Cherr, G. N., and W. H. Clark, Jr. 1983. Characterization of the natural inducers of the acrosome reaction in sturgeon sperm. *J. Cell. Biol.* 97:180a.

Clark, W. H., Jr., A. I. Yudin, F. J. Griffin, and K. Shigekawa. 1984. The control of gamete activation and fertilization in the marine Penaeidae, *Sicyonia ingentis.* In *Advances in Invertebrate Reproduction.* W. Engles, W. H. Clark, A. Fischer, P. J. W. Olive, and D. F. Went, eds. Elsevier, Amsterdam. pp. 459–472.

Conklin, D. E., L. R. D'Abramo, C. E. Bordner, and N. A. Baum. 1980. A successful purified diet for the culture of juvenile lobsters: The effect of lecithin. *Aquaculture* 21:243–249.

Desjardins, L. M., J. D. Castell, and J. C. Kean. 1985. Synthesis of dehydroascorbic acid by subadult lobsters *(Homarus americanus). Can. J. Fish. Aquat. Sci.* 42:370–373.

Downer, R. G. H., and H. Laufer. 1983. *Invertebrate Endocrinology,* vol. 1. Alan R. Liss, New York.

Epel, D., and V. D. Vacquier. 1978. Membrane fusion events during invertebrate fertilization. *Cell Surface Rev.* 5:1–63.

Ferezou, J. P., J. Berreur-Bonnenfant, A. Tekitek, M. Rojas, M. Barbier, S. Wipf, H. K. Wipf, and J. J. Meusy. 1977. Biologically active lipids from the androgenic gland of the crab. In *Marine Natural Products Chemistry.* D. J. Faulkner and W. H. Fenical, eds. Plenum Press, New York. pp. 361–366.

Glabe, C. G., and W. J. Lennarz. 1979. Species-specific sperm adhesion in sea urchins—A quantitative investigation of binding-mediated egg agglutination. *J. Cell Biol.* 83:595–604.

Griffin, F. J., W. H. Clark, Jr., J. H. Crowe, and L. M. Crowe. 1987. Intracellular pH decreases during the *in vitro* induction of the acrosome reaction in the sperm of *Sicyonia ingentis. Biol. Bull.* 173:311–323.

Griffin, F. J., A. I. Yudin and W. H. Clark, Jr. 1985. The effects of a jelly associated protease on the acrosome reaction. *J. Cell Biol.* 101:227a.

Guary, M. M., A. Kanazawa, N. Tanaka, and H. J. Ceccaldi. 1976. Nutritional requirements of prawn. VI. Requirement for ascorbic acid. *Mem. Fac. Fish. Kagoshima Univ.* 25:53–57.

Gwatkin, R. B. L. 1977. Fertilization Mechanisms in Man and Mammals. Plenum Press, New York.

Ikeda, S., and M. Sato. 1964. Biochemical studies on L-ascorbic acid in aquatic animals. III. Biosynthesis of L-ascorbic acid by carp. *Bull. Jpn. Soc. Sci. Fish.* 30:365–369.

Jenness, R., E. C. Birney, and K. L. Ayaz. 1980. Variation of L-gulonolactone oxidase activity in placental mammals. *Comp. Biochem. Physiol.* 67B:195–204.

Kean, J. C., J. D. Castell, and D. J. Trider. 1985. Juvenile lobster (*Homarus americanus*) do not require dietary ascorbic acid. *Can. J. Fish. Aquat. Sci.* 42:368–370.

Lightner, D. V., B. Hunter, P. C. Magarelli, Jr., and L. B. Colvin. 1979. Ascorbic acid: Nutritional requirement and role in wound repair in penaeid shrimp. *Proc. World Maricult. Soc.* 10:513–528.

Lopo, A. C. 1983. Sperm-egg interactions in invertebrates. *In Mechanism and Control of Fertilization.* J. F. Hartman, ed. Academic Press, New York. pp. 269–324.

Lovell, R. T. 1984. Ascorbic acid metabolism in fish. In *Ascorbic Acid in Domestic Animals. I. Wegger, F. J. Tagwerker and J. Moustgaard, eds. Royal Danish Agricultural Society, Copenhagen. pp. 196–205.*

Mantel, L. H., and L. L. Farmer. 1983. Osmotic and ionic regulation. In The Biology of the Crustacea. L. H. Mantel, ed. Academic Press, New York. pp. 53–161.

Milliken, M. R. 1982. Qualitative and quantitative nutrient requirements of fishes: A review. *Fish. Bull.* 80:655–686.

Nishikimi, M., E. Noguchi, and K. Yagi. 1978. Occurrence in yeast of L-galactonolactone oxidase which is similar to a key enzyme for ascorbic acid biosynthesis in animals, L-gulonolactone oxidase. *Arch. Biochem. Biophys.* 191:479–486.

Sandnes, K. 1984. Some aspects of ascorbic acid and reproduction in fish. In *Ascorbic Acid in Domestic Animals.* I. Wegger, F. J. Tagwerker, and J. Moustgaard, eds. Royal Danish Agricultural Society, Copenhagen. pp. 206–212.

Sato, M., R. Yoshinaka, Y. Yamamoto and S. Ikeda. 1978. Nonessentiality of ascorbic acid in the diet of carp. *Bull. Jpn. Soc. Sci. Fish.* 44:1151–1156.

Sato, P., and S. Udenfriend. 1978. Scurvy-prone animals, including man, monkey, and guinea pig, do not express the gene for gulonolactone oxidase. *Arch. Biochem. Biophys.* 187:158–162.

Sato, P., M. Nishikimi and S. Udenfriend. 1976. Is L-gulonolactone-oxidase the only enzyme missing in animals subject to scurvy? *Biochem. Biophys. Res. Commun.* 71:293–299.

Schmell, E. D., and B. J. Gulyas. 1980. Mammalian sperm-egg recognition and binding *in vitro.* I. Specificity of sperm interactions with live and fixed eggs in homologous and heterologous inseminations of hamster, mouse, and guinea pig oocytes. *Biol. Reprod.* 23:1075–1085.

Schneiderman, H., and L. Gilbert. 1958. Substances with juvenile hormone activity in Crustacea and other invertebrates. Biol. Bull. 115:530–535.

SeGall, G. K., and W. J. Lennarz. 1979. Chemical characterization of the component of the jelly coat from sea urchin eggs responsible for induction of the acrosome reaction. *Dev. Biol.* 71:33–48.

Soliman, A. K., K. Jauncey, and R. J. Roberts. 1985. Qualitative and quantitative identification of L-gulonolactone oxidase activity in some teleosts. *Aquacult. Fish. Management* 1:249–256.

Summers, R. G., and B. L. Hylander. 1976. Primary gamete binding: Quantitative determination of its specificity in echinoid fertilization. *Exp. Cell Res.* 100:190–194.

Thomas, P., M. B. Bally, and J. M. Neff. 1985. Influence of some environmental variables on the ascorbic acid status of mullet, *Mugil cephalus* L., tissues. II. Seasonal fluctuations and biosynthetic ability. *J. Fish Biol.* 27:47–57.

Wallace, C. A., R. Jenness, R. J. Mullin, and W. S. Herman. 1985. L-Gulonolactone oxidase is present in

the invertebrate, *Limulus polyphemus*. *Experientia* 41:485–486.
Wilson, R. P. 1973. Absence of ascorbic acid synthesis in channel catfish and blue catfish. *Comp. Biochem. Physiol.* 46B:635–638.
Yamamoto, Y., M. Sato, and S. Ikeda. 1978. Existence of L-gulonolactone oxidase in some teleosts. *Bull. Jpn. Soc. Sci. Fish.* 44:775–779.

Publications

Anchordoguy, T., J. H. Crowe, F. J. Griffin, and W. H. Clark, Jr. 1988. Cryopreservation of sperm from the marine shrimp *Sicyonia ingentis*. *Cryobiology* 25:238–243.
Anderson, S. L., W. H. Clark, and E. S. Chang. 1985. Multiple spawning and molt synchrony in a free spawning natantian (*Sicyonia ingentis*: Penaeidae). *Biol. Bull.* 168:377–394.
Bordner, C. E., L. R. D'Abramo, D. E. Conklin, and N. A. Baum. 1986. Development and evaluation of diets for crustacean aquaculture. *J. World Aquacult. Soc.* 17:44–51.
Borst, D. W., H. Laufer, M. Landau, E. S. Chang, W. A. Hertz, F. C. Baker, and D. A. Schooley. 1987. Methyl farnesoate (MF) and its role in crustacean reproduction and development. *Insect Biochem.* 17(7):1123–1127.
Castell, J. D., D. E. Conklin, J. S. Craigie, S. P. Lall, and K. Norman-Boudreau. 1986. Aquaculture nutrition. In *Realism in Aquaculture: Achievements, Constraints, Perspectives*. M. Bilio, H. Rosenthal, and C. J. Sindermann, eds. European Aquaculture Society, Bredene, Belgium. pp. 251–308.
Chang, E. S. 1985. Hormonal control of molting in decapod Crustacea. *Amer. Zool.* 25:179–185.
Chang, E. S. 1985. Cellular juvenile hormone binding proteins. *Meth. Enzymol.* 111:494–509.
Chang, E. S., M. J. Bruce, and G. D. Prestwich. 1985. Further characterization of the juvenile hormone binding protein from the cytosol of a *Drosophila* cell line: Use of a photoaffinity label. *Insect Biochem.* 15:197–204.
Chang, E. S., M. J. Bruce, and R. W. Newcomb. 1987. Purification and amino acid composition of a peptide with molt-inhibiting activity from the lobster, *Homarus americanus*. *Gen. Comp. Endocrinol.* 65:56–64.
Chang, E. S., and J. D. O'Connor. 1988. Crustacea: Molting. In *Endocrinology of Selected Invertebrates*. R. Downer and H. Laufer, eds. Alan R. Liss, Inc., New York. pp 259–278.
Clark, W. H., Jr., A. I. Yudin, and F. J. Griffin. 1985. Gamete interaction in the penaeidae *Sicyonia ingentis*. *Dev. Growth Differ.* 27:174.
Clark, W. H., Jr., A. I. Yudin, K. J. Shigekawa, and F. J. Griffin. 1986. Cellular events of fertilization in the Penaeidae, *Sicyonia ingentis*. In *Biology of Benthic Marine Organisms*. M.-F. Thompson, R. Sarojini, and R. Nagabushanam, eds. Oxford & IBH, New Dehli. pp. 143–149.
Criddle, K. R., and E. S. Chang. 1985. Effects of container transparency and reflectivity upon oxygen consumption of lobsters. *Aquaculture* 47:373–376.
D'Abramo, L. R., N. A. Baum, C. E. Bordner, D. E. Conklin and E. S. Chang. 1985. Diet-dependent cholesterol transport in the American lobster. *J. Exp. Mar. Biol. Ecol.* 87:83–96.
D'Abramo, L. R., and D. E. Conklin. 1985. Lobster aquaculture. In *Crustacean and Mollusk Aquaculture in the United States*. J. V. Huner and E. E. Brown, eds. AVI Publishing, Westport, Connecticut. pp. 159–201.
D'Abramo, L. R., J. S. Wright, K. H. Wright, C. E. Bordner, and D. E. Conklin. 1985. Sterol requirement of cultured juvenile crayfish, *Pacifastacus leniusculus*. *Aquaculture* 49:245–255.
Goodman, W. E., and E. S. Chang. 1985. Juvenile hormone cellular and hemolymph binding proteins. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 7. G. A. Kerkut and L. I. Gilbert, eds. Pergamon Press, Oxford. pp. 491–510.
Griffin, F. J., and W. H. Clark, Jr. 1987. *In vitro* induction of the acrosome reaction in sperm of the penaeid shrimp *Sicyonia ingentis*. Presented at meeting of World Aquaculture Society, Guayaquil, Ecuador. Abstract.
Griffin, F. J., W. H. Clark, Jr., J. H. Crowe and L. M. Crowe. 1987. Intracellular pH decreases during the *in vitro* induction of the acrosome reaction in the sperm of *Sicyonia ingentis*. *Biol. Bull.* 173:311–323.
Griffin, F. J., K. Shigekawa, and W. H. Clark, Jr. 1988. Formation and structure of the acrosomal filament in the sperm of *Sicyonia ingentis*. *J. Exp. Zool.* 246:94–102.
Griffin, F. J., A. I. Yudin, and W. H. Clark, Jr. 1985. The effects of a jelly associated protease on the acrosome reaction. *J. Cell Biol.* 101:227a.
Hertz, W. A., and E. S. Chang. 1986. Juvenile hormone effects on metamorphosis of lobster larvae. *Intl. J. Inverteb. Reprod. Dev.* 10:71–78.
Jackson, S. A., M. J. Bruce, E. S. Chang, and J. S. Clegg. 1987. Effects of eyestalk ablation upon water relations in the American lobster, *Homarus americanus*. *J. Exp. Zool.* 244:289–393.
Kean, J. C., J. D. Castell, A. G. Boghen, L. R. D'Abramo, and D. E. Conklin. 1985. A reevaluation of the lecithin and cholesterol requirements of juvenile lobster (*Homarus americanus*) using crab protein-based diets. *Aquaculture* 47:143–149.
Lynn, J. W., and W. H. Clark, Jr. 1987. Physiological and biochemical investigations of the egg jelly release in *Penaeus aztecus*. *Biol. Bull.* 173:451–460.
Nelson, K., B. Heyer, E. Johnson, D. Hedgecock, and E. S. Chang. 1988. Photoperiod-induced changes in hemolymph vitellogenins in female lobsters (*Homarus americanus*). *Comp. Biochem. Physiol.* 90B(4):809–821
Norman-Boudreau, K., and D. E. Conklin. 1985. Antinutritional properties of egg white in diets for lobsters. *Bull. Jpn. Soc. Sci. Fish.* 51:953–957.
Norman-Boudreau, K., and D. E. Conklin. 1986. Purified proteins in artificial diets for juvenile lobsters. In *Proceedings of the 1st International Conference on Warm Water Aquaculture-Crustacea*. G. L. Rogers, R. Day, and A. Lim, eds. Brigham Young Univ., Laie, Hawaii. pp. 343–351.
Okazaki, R. K., M. J. Snyder, and E. S. Chang. 1988. Ecdysteroids in nemerteans: Presence and physiological role. *Hydrobiologia.* 156:153–160.
Pillai, M. C., and W. H. Clark, Jr. 1987. Oocyte activation in the marine shrimp, *Sicyonia ingentis*. *J. Exp. Zool.* 244:325–329.
Prestwich, G. D., J. K. Koeppe, J. J. Brown, E. S. Chang, and A. K. Singh. 1985. Experimental techniques for photoaffinity labelling of juvenile hormone binding proteins of insects with epoxyfarnesyl diazoacetate (EFDA). *Meth. Enzymol.* 111:509–530.
Shigekawa, K., and W. H. Clark, Jr. 1986. Spermiogenesis in the marine prawn, *Sicyonia ingentis*. *Dev. Growth Differ.* 28:95–112.
Snyder, M. J., and E. S. Chang. 1984. Regulation of ecdysteroid titers and

molting periodicity in larvae of the American lobster, *Homarus americanus*. *Amer. Zool.* 24:33a.

Snyder, M. J., and E. S. Chang. 1986. Effects of eyestalk ablation on larval molting rates and morphological development of the American lobster, *Homarus americanus*. *Biol. Bull.* 170:232–243.

Snyder, M. J., and E. S. Chang. 1986. Effects of sinus gland extracts on larval molting and ecdysteroid titers of the American lobster, *Homarus americanus*. *Biol. Bull.* 170:244–254.

Trevelyan, G. T., and E. S. Chang. 1987. Light-induced shell pigmentation in post-larval *Mytilus edulis* L. and its use as a biological tag. *Mar. Ecol. Prog. Ser. 39:137–144.*

Theses

Griffin, F. J. 1987. Induction and control of the acrosome reaction in the sperm of *Sicyonia ingentis*. Doctoral dissertation, University of California, Davis.

Norman-Boudreau, K. 1986. Qualitative and quantitative requirements for protein in the diet of juvenile lobster (*Homarus* sp.). Doctoral dissertation, University of California, Davis.

Snyder, M. J. 1985. Endocrine interactions during larval development of the American lobster, *Homarus americanus* (Crustacea, Decapoda). Master's thesis, University of California, Davis.

Lectures and Conferences

Chang, E. S. Insect hormone receptors: Regulation of insect gene expression by juvenile hormone and ecdysone. Colorado State University, Ft. Collins, July 1985. Invited speaker.

Chang, E. S. Regulation of larval ecdysteroid titers and ecdysis in the American lobster (*Homarus americanus*). 10th International Symposium on Comparative Endocrinology, Copper Mountain, Colorado, July 1985. Invited speaker.

Chang, E. S. Molt-inhibiting hormone from the eyestalks of the lobster, *Homarus americanus*. World Mariculture Society, Reno, Nevada, January 1986.

Chang, E. S. Western Regional Conference on Comparative Endocrinology. University of California, Davis, March 1986.

Chang, E. S. Peptides, steroids, crustaceans, and insects. Endocrinology Graduate Group, University of California, Davis, May 1986.

Chang, E. S. Crustacean molting: Characterization of the lobster molt-inhibiting hormone. Bodega Marine Laboratory, Bodega Bay, California, August 1986.

Chang, E. S. Research and teaching at the Bodega Marine Laboratory. Coastwalk, Bodega Bay, California, August 1986.

Chang, E. S. Juvenile Hormone Receptors Roundtable. 4th International Symposium on Juvenile Hormone. Niagara-on-the-Lake, Canada, September 1986. Invited speaker.

Chang, E. S. Crustacean molting: Role of peptide and steroid hormones. Department of Biological Sciences, Universtity of California, Santa Barbara, February 1987. Invited speaker.

Clark, W. H. U.S.-Taiwan Workshop. National Academy of Sciences, Honolulu, Hawaii, January 1985. Invited speaker.

Clark, W. H. Induction of the acrosome reaction in sperm of the marine shrimp *Sicyonia ingentis*. Gordon Conference on Fertilization. Plymouth, New Hampshire, August 1985. Invited speaker.

Conklin, D. E. Evolution of ascorbic acid biosynthesis in fish. Bodega Marine Laboratory, Bodega Bay, California, May 1986.

Conklin, D. E. Ascorbic acid biosynthesis: A paradigm shift. Group in Nutrition, University of California, Davis, February 1987.

Cryopreservation of Crustacean Gametes

John H. Crowe

University of California, Davis
R/A-62
Project Initiated: October 1, 1984
Project Completed: September 30, 1987

The shrimp industry is the most valuable fishery in the United States, amounting in 1981 to a dockside value of nearly $1.5 billion. Of this total, only about $509 million was due to domestic catch; the rest was imported from foreign fisheries or aquaculture operations. Demand for shrimp in the United States is rising, and the existing fishery clearly cannot keep up with this demand. As a result, shrimp aquaculture is rapidly emerging as a major industry. Nevertheless, one of the problems in establishing the industry is the availability of gametes to start cultures. Currently, gravid females are caught in the wild and spawned in captivity. Many shrimp species reproduce seasonally, so there is a critical limitation to the times at which cultures can be initiated, thus limiting the industry severely. This project has been aimed at providing solutions to this problem.

Because it is possible to produce ovulating females at any time of year by appropriate hormonal treatment or ablation of the eyestalks, we have concentrated on the limiting gamete, the sperm. In collaboration with W. H. Clark's laboratory, we have developed methods for cryopreservation of the sperm of one commercial species, *Sicyonia ingentis*, that may provide a source of sperm year-round. Furthermore, we have discovered the likely mechanism of activation of the sperm, a finding that may lead to development of means for activating the cells *in vitro*, thus making it possible to improve the fertilizing capabilities of sperm cells that would otherwise not be usable. As our joint findings on activation of the cells is summarized in the report by Clark's group, they will not be discussed here. This final report summarizes the research that led to the discoveries concerning cryopreservation.

Screening Potential Cryoprotectants

At the outset of these studies, we were aware that cryopreservation of shrimp gametes was likely to be difficult; previous studies had limited success with such cells. Thus, we used an unusual procedure in an attempt to identify new cryoprotectants that might be useful in preserving these cells specifically. Because there is general agreement now in the cryobiology community that cell membranes are the most likely site of damage during freezing, we initially used isolated membranes rather than intact cells to study effects of freezing. We isolated Ca^{2+}-transporting membranes from crustacean muscle and used stability of those membranes during freezing and thawing as models for selecting cryoprotectants. We found that it was possible to preserve biological activity in these membranes completely when they were frozen and thawed in solutions containing trehalose, sucrose, or proline (Rudolph and Crowe, 1985). The conventional cryprotectants glycerol and dimethylsulfoxide (DMSO) were decidedly inferior to any of these.

Mechanism of Cryoprotection

In an effort toward developing even more effective cryoprotectants, we have studied extensively the mechanism by which they stabilize membranes. The results of those studies have shown that the molecules that are effective have two major functions: They inhibit fusion between phospholipid bilayers during freezing and thawing, and by direct interaction with either the polar head group or hydrocarbon chains of the phospholipids, they alter the phase properties of the lipid.

Fusion. Using resonance energy transfer between fluorescent probes included in the bilayer to assay fusion, we were able to show that the molecules that preserve the dry membranes also are effective at inhibiting fusion during freeze-thaw (Womersley et al., 1986; Rudolph et al., 1986).

Effects on Phase Properties. If phospholipids pass through their gel to liquid crystalline phase transition, the bilayer in which they are contained increases its permeability. As a result, cell contents are leaked to the surrounding medium. Thus, effective cryoprotectants might be expected to alter the phase properties of phospholipids, and they do (Crowe et al., 1985; Rudolph et al., 1986). The transition temperature is driven down by effective cryoprotectants, thus inhibiting the transition.

Modes of Interaction Between Cryoprotectants and Phospholipids. We have investigated the mechanism of interaction that may lead to stabilization, with the following results. A major class of cryprotectants, the disaccharides sucrose and trehalose, unquestionably interacts directly with the polar head group of phospholipids, probably by hydrogen bonding between -OH groups on the sugars and the phosphate of the phospholipid. This interaction forces the head groups apart, as a result of which interactions between the hydrocarbon chains are inhibited, and the lipids do not enter gel phase readily. Several lines of evidence support this mechanism (Rudolph et al., 1986; Crowe et al., 1987a; Anchordoguy et al., 1987). Other molecules containing hydrophobic residues that are effective cryoprotectants interact with the bilayer by distinctly different

mechanisms. The best known molecule in this category is proline. The hydrophobic ring of this molecule spontaneously associates in solution, forming stacks with the carboxyl groups oriented into the aqueous phase (Rudolph and Crowe, 1986). These stacks of proline molecules in turn associate with the hydrocarbon chains of phospholipids by hydrophobic interaction. Because this interaction is less stable than the hydrogen-bonded association between sugars and the polar head groups of phospholipids, proline can, under certain conditions, be forced out of the bilayer, which may make it unsuitable as a cryoprotectant (Rudolph et al., 1986).

Cryoprotection of Shrimp Sperm

Armed with the information about cryoprotection obtained from studies on model systems, we next began studies on cryoprotection of intact sperm of the shrimp *S. ingentis*. This shrimp was chosen specifically because W. H. Clark's research group, with whom we collaborate extensively, had previously developed bioassays for determining the viability and fertilizing capacity of these sperm. This is the only shrimp species for which these assays are available. As described in Clark's report, these shrimp undergo complex activation events that provide an ideal assay for their viability. Furthermore, Clark's group has discovered ways in which the cells can be induced to undergo these events *in vitro*, so the cells can be frozen and thawed and their ability to undergo activation can subsequently be studied.

In our initial studies, we used the morphology of the cells to screen potential cryoprotectants further. On the basis of morphological damage from freezing, we were able to show that trehalose, sucrose, proline, glycerol, and DMSO were all able to preserve the morphology about equally effectively (Anchordoguy et al., 1988). However, the most effective of these in terms of preserving the ability to undergo activation events was DMSO. In the first experiments along these lines, we found that DMSO was capable of preserving about 30% of the sperm after freezing in liquid nitrogen, whereas the other cryoprotectants, which we had shown to be more effective than DMSO *in vitro*, yielded only about 16% preservation.

Because DMSO is known to cross biological membranes, we suspect that its effectiveness is related to its ability to enter the cytoplasm of the cell. Thus, even though it is inferior to other cryoprotectants *in vitro*, it is the most effective with the intact cells. It follows, however, that if we can introduce molecules like trehalose and sucrose into the cytoplasm at high concentrations cryopreservation may be improved significantly. So far, all studies with this aim in view have involved using DMSO itself to make the plasma membrane permeable. This molecule is known to increase permeability, so we reasoned that it may be possible to use it to introduce another, more effective, cryoprotectant into the cytoplasm. All experiments along these lines have so far failed. We think it will be possible to use other techniques, particularly electropermeabilization, however, to make the cells permeable. If we are successful in this regard, we strongly suspect that cryopreservation can be improved significantly.

In view of the fact that DMSO has been the most effective cryoprotectant we have tested with these cells, albeit for the aforementioned reasons, we have developed an interim method for cryopreserving the sperm that will be at least minimally acceptable to industry. Through much trial-and-error experimentation on the rate of cooling, temperature at which ice nucleation is commenced during freezing, and the way in which the DMSO is introduced to the cells, we have developed a method that yields 60–70% survival of these sperm after cryopreservation. The cells have been kept frozen for at least 90 days without serious loss of viability (Anchordoguy et al., 1988).

We view the method involving DMSO as only an interim solution for the following reasons. DMSO is toxic to the cells in the unfrozen state. As a result, it is necessary to remove the DMSO rapidly after they are thawed, an inconvenience in an industrial setting. In addition, the cooling protocol must be followed rigorously to obtain good results, and it requires expensive equipment that again is undesirable for industry. With these objections in mind, and with renewed support from Sea Grant, we are continuing to search for a suitable method for introducing more effective cryoprotectants into these cells.

References

Anchordoguy, T. J., J. H. Crowe, F. J. Griffin, and W. H. Clark, Jr. 1988. Cryopreservation of sperm from the marine shrimp, *Sicyonia ingentis*. *Cryobiology* 25:238–243.

Anchordoguy, T. J., A. S. Rudolph, J. F. Carpenter, and J. H. Crowe. 1987. Modes of interaction of cryoprotectants with membrane phospholipids during freezing. *Cryobiology* 24:324–331.

Crowe, J. H., L. M. Crowe, J. F. Carpenter, and C. A. Wistrom. 1987a. Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochem. J.* 242:1–10.

Crowe, J. H., B. J. Spargo, and L. M. Crowe. 1987b. Preservation of dry liposomes does not require retention of residual water. *Proc. Natl. Acad. Sci.* 84:1537–1540.

Crowe, L. M., J. H. Crowe, A. Rudolph, C. Womersley, and L. Appel. 1985. Preservation of freeze-dried liposomes by trehalose. *Arch. Biochem. Biophys.* 242:240–247.

Griffin, F. J., W. H. Clark, Jr., J. H. Crowe, and L. M. Crowe. 1987. Intracellular pH decreases during the *in vitro* induction of the acrosome reaction in the sperm of *Sicyonia ingentis*. *Biol. Bull.* 173:311–323.

Rudolph, A. S., and J. H. Crowe. 1985. Membrane stabilization during freezing: The role of two natural cryoprotectants, trehalose and proline. *Cryobiology* 22:367–377.

Rudolph, A. S., and J. H. Crowe. 1986. A calorimetric and infrared spectroscopic study of the stabilizing solute proline. *Biophys. J.* 50:423–430.

Rudolph, A. S., J. H. Crowe, and L. M. Crowe. 1986. Effects of three

stabilizing agents—proline, betaine, and trehalose—on membrane phospholipids. *Arch. Biochem. Biophys.* 245:134–143.
Womersley, C., P. S. Uster, A. S. Rudolph, and J. H. Crowe. 1986. Inhibition of dehydration-induced fusion between liposomal membranes by fluorescence energy transfer. *Cryobiology* 23:245–255.

Publications

Anchordoguy, T. J., J. H. Crowe, F. J. Griffin, and W. H. Clark, Jr. 1988. Cryopreservation of sperm from the marine shrimp, *Sicyonia ingentis*. *Cryobiology* 25:238–243.
Anchordoguy, T. J., A. S. Rudolph, J. F. Carpenter, and J. H. Crowe. 1987. Modes of interaction of cryoprotectants with membrane phospholipids during freezing. *Cryobiology* 24:324–331.
Crowe, J. H., L. M. Crowe, J. F. Carpenter, and C. A. Wistrom. 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochem. J.* 242:1–10.
Crowe, J. H., B. J. Spargo, and L. M. Crowe. 1987. Preservation of dry liposomes does not require retention of residual water. *Proc. Natl. Acad. Sci.* 84:1537–1540.
Crowe, L. M., J. H. Crowe, A. Rudolph, C. Womersley, and L. Appel. 1985. Preservation of freeze-dried liposomes by trehalose. *Arch. Biochem. Biophys.* 242:240–247.
Griffin, F. J., W. H. Clark, Jr., J. H. Crowe, and L. M. Crowe. 1987. Intracellular pH decreases during the *in vitro* induction of the acrosome reaction in the sperm of *Sicyonia ingentis*. *Biol. Bull.* 173:311–323.
Rudolph, A. S., and J. H. Crowe. 1985. Membrane stabilization during freezing: The role of two natural cryoprotectants, trehalose and proline. *Cryobiology* 22:367–377.
Rudolph, A. S., and J. H. Crowe. 1986. A calorimetric and infrared spectroscopic study of the stabilizing solute proline. *Biophys. J.* 50:423–430.
Rudolph, A. S., J. H. Crowe, and L. M. Crowe. 1986. Effects of three stabilizing agents—proline, betaine, and trehalose—on membrane phospholipids. *Arch. Biochem. Biophys.* 245:134–143.
Womersley, C., P. S. Uster, A. S. Rudolph, and J. H. Crowe. 1986. Inhibition of dehydration-induced fusion between liposomal membranes by fluorescence energy transfer. *Cryobiology* 23:245–255.

Absorption of Nutrients by Fish

University of California Medical School, Los Angeles
R/A-63
Project Initiated: October 1, 1985
Project Completed: September 30, 1987

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

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

In previous project years, we had studied how eight fish species (rainbow trout, channel catfish, common carp, tilapia, grass carp, sturgeon, striped bass, and monkey-faced prickleback) differed in the capacities of their intestines to absorb nutrients while all fish were being maintained in our laboratory on the same artificial diet. The interest of this experiment stemmed from the fact that these fish species have different natural diets: three (common carp, grass carp, and tilapia) are herbivores, two (catfish and sturgeon) are omnivores, two (striped bass and trout) are carnivores, and one (monkey-faced prickleback) is carnivorous when small but herbivorous when large. The natural diets of herbivores are relatively high in carbohydrate and low in protein, whereas the natural diets of carnivores are relatively high in protein and low in carbohydrate. Experiments with vertebrate species other than fish had established that in omnivorous species the absorptive capabilities of the intestines adapt rapidly to their diets, by increasing sugar absorption when a high-carbohydrate diet is eaten and by increasing amino acid absorption when a high-protein diet is eaten. By maintaining all eight species in our laboratory on the same artificial diet, we tested whether any genetic differences in intestinal absorption among these fish species are related to their different natural diets and whether any reversible adaptation to diet occurs within the lifetime of a fish.

This experiment established that the ratio of amino acid transport to glucose transport was highest in carnivores, intermediate in omnivores, and lowest in herbivores. Most of this variation was due to species differences in glucose uptake rather than in proline uptake. Thus, these species differences are genetic ones that natural selection has programmed into each species in response to its normal natural diet.

We then proceeded to study the function of pyloric caeca in fish. Many, but not all, species of fish have dead-end tubes that branch off the proximal intestine and that are termed pyloric caeca. The existence of the caeca has been well known since the time of Aristotle, but their function has been debated. Various proposed functions have included fermentation, food storage, fat digestion, enzymatic digestion, and nutrient absorption. However, there was almost no direct information about the absorptive function of the pyloric caeca until recently, when Collie studied their function in Coho Salmon.

We chose four fish species that differ nearly 40-fold in mean number of cacea: cod, rainbow trout, largemouth bass, and striped bass; these have on the average 222, 56, 25, and 6 caeca, respectively. For each fish species we measured absorption of the amino acid proline and of the sugar glucose along the caeca and along the intestine, and we measured the length, diameter, thickness, and surface area of both the caeca and the intestine. In trout we carried out further measurements: absorption of nine other amino acids plus the dipeptide carnosine; rates of filling and emptying of the caeca, as monitored by X-ray studies after ingestion of a radio-opaque marker; use of glass beads of different sizes to determine the maximal size of particle that can enter the caeca; and activities of membrane-bound hydrolytic enzymes.

In all four species we found that the caeca absorbed amino acid and sugar at rates comparable with rates in the proximal intestine, as expressed in transport rate per milligram or per square centimeter of tissue. Thus, the proportion that the caeca contribute to the absorptive capacity of the whole gut is almost directly proportional to the proportion that the caeca contribute to the surface area of the whole gut. In the two species with the most numerous caeca, the cod and rainbow trout, the caeca account for more than half of the entire gut's absorptive capacity for sugars and amino acids. The proportion is around 50% in largemouth bass and is about 12% in striped bass. In tuna, in which the caeca are fused and much more extensive, their contribution to the gut's total absorptive capacity is probably even higher, in excess of 90%.

In trout we showed that the caeca fill and empty with the same time course as the small intestine, that they only admit particles with a diameter smaller than 150μm, and that they possess disaccharidase

and dipeptidase activities.

Because the caeca fill and empty at the same rate as the intestine, they cannot serve as sites for food storage or of delayed fermentation.

In short, the caeca play an important role in digestion and absorption of carbohydrate and protein. In cod, trout, and probably some other species, including tuna, the caeca are in fact the main site of nutrient absorption.

Our next goal was to study how nutrient uptake rates vary phenotypically with changes in dietary nutrient levels. Recall that the carp is an omnivore that normally does encounter changes in dietary nutrient inputs, whereas the trout is a strict carnivore whose dietary proportions of protein and carbohydrate normally undergo little change. We studied intestinal absorption in both species after they had spent many weeks or months eating a diet devoid in carbohydrate but high in protein or a diet with moderate levels of both carbohydrate and protein. The carp adapted to high dietary carbohydrate levels by increasing intestinal sugar absorption. The trout did not exhibit such adaptation. Thus, natural selection has programmed into the carp the capacity to vary its intestinal absorption, but has not programmed this capacity in the trout, which would not normally encounter significant amounts of dietary carbohydrate.

We then determined, for trout and catfish as well as for prickleback, how intestinal adaptation to diet varies with the age of the fish. The most marked variation was observed in catfish and prickleback, which are largely carnivorous when small and become increasingly herbivorous with age. We studied individuals of various sizes (ages), all maintained in the laboratory on the same diet. In both species the larger animals had lower amino acid uptake rates relative to glucose uptake rates than did smaller animals. These differences in intestinal function correspond to the dietary differences that these two species would normally encounter: a diet high in amino acids in smaller fish and a diet high in sugars in larger fish. Thus, the intestine of each species is adapted to absorb best the nutrients that each species would normally encounter at that age. However, these differences exist even in the absence of the usual age-related signal of the changing diet itself. Thus, these developmental changes in intestinal function must be genetically "hard-wired," that is, genetically programmed into the animal and not dependent on input of dietary solutes as a signal.

Our final experiment consisted of examining what amino acids, as a dietary supplement, were most effective at inducing uptake of amino acids. The intestine has several different transporters for different classes of amino acids: one for acidic amino acids, another for basic amino acids, another for neutral amino acids, and still another for the amino acid proline. We naively expected that each transporter would be best induced by dietary supplementation with the particular amino acid(s) that it transports. This expectation was partly satisfied: the transporter of acidic amino acids was best stimulated by the acidic amino acid aspartate, whereas the transporter of basic amino acids was stimulated by the basic amino acid lysine. However, unexpected discrepancies also occurred. For example, aspartate induced the transporter of basic amino acids even better than did lysine, whereas proline was not an especially effective inducer of the transporter of this amino acid. Thus, in considering amino acids as dietary supplements, one must consider not only the role of the amino acid as a possibly essential nutrient but also its ability to stimulate absorption of other amino acids.

Cooperating Organizations

Bioproducts, Inc.
Star Milling Company
The Fishery
Fish Breeders
Pacific Aqua Farms
Calaqua
Whitewater Trout Company
Widman Fish Farm
California State Department of Fish and Game
Coachella Valley Water District
Institute of Marine Biochemistry, Aberdeen, Scotland

Publications

Buddington, R. K., and J. M. Diamond. 1985. Diet dependence of vertebrate intestinal nutrient transport: A genotypic component. *Fed. Proc.* 44:811.

Buddington, R. K., and J. M. Diamond. 1986. Aristotle revisited: The function of pyloric caeca in fish. *Proc. Natl. Acad. Sci.* 83:8012–8014.

Buddington, R. K., and J. M. Diamond. 1986. Pyloric ceca of fish, a "new" absorptive organ. *Am. J. Physiol.* 252:G65–G76.

Buddington, R. K., J. Chen, and J. M. Diamond. 1985. Genotypic regulation of vertebrate intestinal nutrient transport. *Abstr. Amer. Assoc. Adv. Sci.* 134.

Buddington, R. K., J. W. Chen, and J. M. Diamond. Evolution of constraints on the adaptive flexibility of nutrient transport by vertebrate intestine. Presented at annual meeting of the American Physiological Society, Anaheim, California, October 1987.

Buddington, R. K., J. W. Chen, and J. M. Diamond. 1987. Genetic and phenotypic adaptation of intestinal nutrient absorption to diet in fish. *J. Physiol.* 393:261–281.

Diamond, J. M. and R. K. Buddington. 1985. Pyloric caeca: A "new" absorptive organ. *Fed. Proc.* 44:811.

Diamond, J. M., and R. K. Buddington. 1987. Intestinal nutrient absorption in herbivores and carnivores. In *Comparative physiology: Life in water and on land.* P. Dejours, L. Bolis, C. R. Taylor, and E. R. Weibel, eds. Springer-Verlag, New York. pp. 193–203.

Karasov, W. H., R. K. Buddington, and J. M. Diamond. 1985. Adaptation of intestinal sugar and amino acid transport in vertebrate evolution. In *Transport processes, iono-, and osmoregulation.* R. Gilles and M. G. Gilles-Baillien, eds. Springer-Verlag, Berlin. pp. 227–239.

Saul-Conn, C. and J. M. Diamond. 1985. Nutrient absorption by fish. *Abstr. Amer. Assoc. Adv. Sci.* 130.

Lectures

Buddington, R. K. Intestinal nutrient absorption. Saint John's University, New York, March 1987.

Buddington, R. K. Intestinal nutrient absorption. University of Lowell, Massachusetts, May 1987.
Buddington, R. K. Intestinal nutrient absorption. University of Cambridge, England, September 1987.
Buddington, R. K. Intestinal nutrient absorption. University of Bergen, Norway, September 1987.
Buddington, R. K. Intestinal nutrient absorption. University of Tromso, Norway, September 1987.
Diamond, J. M. Intestinal nutrient absorption. University of California, San Diego, December 1986.
Diamond, J. M. Intestinal nutrient absorption. University of Pretoria, South Africa, February 1987.
Diamond, J. M. Intestinal nutrient absorption. FASEB Symposium, Copper Mountain, Colorado, July 1987.

Development of Pacific Oyster Broodstock

Dennis Hedgecock

University of California, Davis
Bodega Marine Laboratory
R/A-65
Project Initiated: October 1, 1987
Project Completed: September 30, 1988

California's oyster industry is based on extensive culture of the Pacific oyster (*Crassostrea gigas*), an introduced species that does not reproduce locally. In the early 1980s, this industry became dependent on seed produced by a few commercial hatcheries and shipped to west coast growers as eyed-larvae for on-site setting (Chew, 1984). Short-term problems with this method (poor setting success, lack of control over seed quality) as well as long-term opportunities for genetic improvement in traits such as growth rate and resistance to summer mortality (Hershberger et al., 1984) were addressed in this project.

Development of Systems for Experimental Culture

Genetic experiments with oysters require the raising of many family groups in necessarily small volumes of water. Progress toward our objectives has thus depended on development of the capability to condition and spawn brood stock, to make controlled crosses, to rear families in separate cultures to metamorphosis, and to nurse these pedigreed spat to a seed size suitable for transfer to nearby commercial oyster beds. Our early attempts at small-volume larval culture saw few families reared to seed size. The frequent "crashes" of small-volume cultures was quite different than the deaths experienced in large volumes (Hedgecock, 1985a).

In the course of establishing capability, therefore, we had first to understand the causes of oyster mortality in our culture systems. Work that repeatedly pointed to microbial fouling as a significant cause of larval mortality in small-volume culture led to the development of a polyethylene-bag culture and husbandry system that consistently yields a final density of about one to two eyed larvae per milliliter as reported last year (Hedgecock, 1987).

The chief advantages of this system are (1) water circulation within bags that keeps larvae from remaining long in contact with surfaces where they pick up epibiotic microbial fouling; (2) reduced exposure to airborne microorganisms implicated as a source of contamination; (3) easy maintenance of a large number of larval cultures; (4) light transmission to the culture water so that algal cells continue to divide, keeping a low, steady concentration of algal food and (5) reduced variance in larval size.

We earlier had reported that larval developmental rate to metamorphosis was inversely related to larval density and survival (Hedgecock, 1987). This year a graduate student in our laboratory, Marilyn Thompson, showed that time to metamorphosis was independent of larval density after 12 days postspawn, although densities of >4/ml adversely affected survival. Thus, by adjusting larval density after differential early mortality among families, we can reduce the variance among families and treatments in time to metamorphosis and make their developmental histories more comparable.

This year we discovered still another major advantage of bag culture. Last year we showed that epinephrine (Coon et al., 1985) can be added directly to a bag culture to induce metamorphosis, mostly without attachment (Hedgecock, 1987). This year further experiments showed that treatment with epinephrine was unnecessary because larvae that attach to the bag are easily removed by gentle brushing with a camel's hair brush with little mortality. A preliminary experiment last year gave strong indication that postmetamorphic growth of spat circulating in these bags is much greater than growth of spat attached either to the bag walls or to cultch material in buckets. This was confirmed this year in an experiment by a work-study student, Marc Roach. He found significantly greater growth of unattached spat in otherwise comparable conditions.

We previously described and illustrated the large upwelling systems used to rear spat to seed size (Hedgecock, 1986). To review, this system holds a total of 228 individual upwelling units made from 3-in. (7.6 cm) polyvinylchloride cylinders. Growth and survival of spat is uniform over surprisingly broad ranges of spat density, algal density, and flow rate. Fifty-four full-sib families (most within half-sib groups sharing a common parent) have been reared in this system, although in previous years some were accidently lost before they could be transferred to the field for grow-out. Grow-out of progenies produced in our hatchery and nursery systems has been done in commercial beds in Tomales Bay as well as on a rack located within the Marine Reserve in Bodega Harbor. Survival and growth of these progenies have been excellent, and some have been brought back to the hatchery for successful F_2 crosses. The factorial and heirarchial mating experiments accomplished thus far may now permit partitioning of variation in survival and growth, but the clear differences among full- and half-sib groups already reinforce the value in making more of such crosses.

Ploidy Manipulation Experiments

In the first year of the project, we did one experiment in which we

used cytochalasin B (CB) to induce triploidy by blocking the second meiotic division (Hedgecock, 1986). Survival to the spat stage of eggs treated with CB was poor (0.001%). In the second year, we tested alternatives to CB in an effort to find an inhibitor of cell division that would be as effective but less toxic. The related inhibitor cytochalasin D holds some promise in this regard, and this year an exchange student from Ecuador, Xavier Orellano, conducted several excellent experiments comparing the two; he showed that only very early mortality was adversely affected by the inhibitor. Future experiments should explore the use of other ways of inhibiting cell division, such as thermal shock (Quillet and Panelay, 1986).

Heterozygosity and Growth Experiment

This year, experiments were designed to test the effects of heterozygosity on growth rate in Pacific oysters. Three treatments were used. Eggs were fertilized normally, and then two of three groups were treated with CB to inhibit the formation of the first and second polar bodies to create triploids. The separate treatment groups will differ in heterozygosity depending on gene-centromere recombination rates at each locus.

In the first experiment, we used 60 brood stock obtained from Hog Island Oyster Co. On the 11th day, the presence of many "D" hinge larvae in the CB containers suggested that CB treatment had retarded larval growth (or possibly that surviving larvae were slow growers). The remaining spat are now being cultured in our nursery system.

In a final experiment, 61 brood stock provided gametes to generate the three treatment groups. CV-1s were treated between 5 and 15 min after fertilization, and CB-2s were treated between 20 and 40 min after fertilization, following a suggestion made by Ken Chew (Lu, 1986). All three treatment groups were subsequently stocked into larval rearing bags at @ 1.5/ml, and remaining CV-1s were caught on cultch. By 42 days after spawning, all were moved into the nursery for grow-out, and at 63 days, animals on cultch were moved into the field. The growth performance of these will be monitored, and their genetic makeup will be determined by allozyme electrophoresis.

Egg Activation

Artificial (aspermic) activation of oocytes is the first step in production of oyster parthenotes. Fred Sly focused on factors that cause a calcium release in the egg and also promote activation of protein kinase C as those most likely to result in aspermic activation. In two experiments, the first a two-factor design treating with calcium ionophore and phorbol ester and the second a 2 x 3 factor design that included washing out the activants at various times, there was no significant activation in the treatment groups when compared with unfertilized control groups. Calcium ionophores and phorbol esters, although active in other systems, did not result in normal activation and development of oyster oocytes.

In all subsequent experiments, the activated and fertilized treatment groups were treated with CB to restore diploidy in activated eggs. In a 2 x 5 design, we treated unfertilized and fertilized eggs with ammonium, Ca^{2+}, ammonium and Ca^{2+}, or 50% seawater (diluted with deionized water). Controls were untreated. Again, there was no significant development in the unfertilized treatment groups when compared with the unfertilized seawater controls.

We substituted oleyl-acetyl-glycerol (OAG) for phorbol esters in a 2 x 2 design that used calcium ionophore as the other treatment. In the second such experiment with adequate controls, the activated treatment groups did not develop significantly. We had similar lack of success with bombesin, 1,2 dioctanoyl-sn-glycerol (c:8) (DOG), and high-calcium seawater (20 mM). In two final experiments, an isotonic (0.3 M) $CaCl_2$ solution was used as an activant. We concluded that an isotonic $CaCl_2$ solution may be an effective oocyte activant. Future experiments will include a schedule of washout and DOG as a treatment. The possibility of restoring diploidy with CB will be investigated further.

Loss of Genetic Diversiy in Hatchery Stocks

Hatchery production of oyster seed has stabilized the seed supply and has met increasing demands. Hatchery production of seed can, however, result in loss of genetic diversity in hatchery stocks because conservation of genetic resources is generally not a consideration in meeting production goals. We (Sly and Hedgecock, 1988) compared two measures of genetic diversity between two third-generation hatchery-propagated populations and the wild stock in Dabob Bay, Washington, from which they were originally derived. The two derived populations were reared separately in Willapa Bay, Washington, and Humboldt Bay, California. We compared average heterozygosities between wild and derived stocks and then calculated average effective population sizes of separate derived stocks by using the temporal variances in allelic frequencies at 14 polymorphic enzyme-coding loci.

Average heterozygosities were not significantly different between the derived populations and the wild stock (Table 1). This result was unexpected because both derived populations show a loss of rare alleles. Loss of rare alleles in subsequent generations is an expected result if derived populations were established from small numbers of breeding individuals. Additional evidence of restricted breeding population size is evident in comparisons of the percentage of polymorphism ($P_{0.95}$), which decreased from 92.8% in the wild stock to 85.7% in the Willapa Bay stock and 64.3% in the Humboldt Bay stock. The average effective population sizes over three generations calculated from the

Table 1. Average Heterozygosities, Percentage of Polymorphisms ($P_{0.95}$), and Variance-Effective Population Sizes of the Willapa and Humboldt Bay Stocks When Compared to the Dabob Bay Stock from Which They Were Derived.

Average Heterozygosities	Dabob Bay	Willapa Bay	Humboldt Bay
Observed	0.262	0.268	0.256
Expected	0.282	0.248	0.252
Percentage of Polymorphism ($P_{0.95}$)	0.928	0.857	0.643
Effective Population Sizes		40.6 (±13.9)	8.7 (±2.1)

variance in allelic frequencies of enzyme-coding loci were 40.6 (± 13.9) and 8.7 (± 2.1) in the Willapa and Humboldt Bay stocks, respectively.

Allelic frequency divergence at the Aat locus in the Humboldt population is greater than expected if genetic drift alone was acting to cause population differentiation, suggesting that a force such as natural selection may also be acting at this locus.

The results show that restrictions in the size of breeding populations cause loss of genetic diversity during hatchery production of seed. We suggest that stock pedigrees be established to control the process of domestication and to eliminate the potential problems associated with loss of genetic diversity (Hedgecock, 1987).

Generation of Oyster Families

An important step in domestication of aquatic species such as oysters is establishing pedigreed lines. Pedigreed lines can be used to evaluate individual performance and to determine the genetic and environmental components of performance. Three sets of crosses have been accomplished in our newly renovated hatchery. All families were reared as larvae and set in 8-l plastic bags that have provided excellent larval survival (Borgeson et al., 1989). The brood stock used to produce all families were Dabob Bay natural-set animals maintained in Tomales Bay, California. In two of the crosses, three males were crossed each with three different females in a hierarchical design for a total of nine families. In the second cross, two males and four females were crossed in all combinations. The parents of each cross were frozen at –70°C for storage before electrophoresis.

In the first cross, all of the families had been moved into the nursery system by 54 days. On the 173rd day after the spawn, the majority of this cross was moved into the field for grow-out on the commercial lease of the Hog Island Oyster Co. in Tomales Bay, California. In the second set of families, each family was divided into two treatment groups, diploids and triploids. Triploids were produced by treating fertilized eggs with CB. The triploid families showed poorer survival than the diploid families, and some triploid families were lost. By 45 days after the spawn, all of the families had been moved into the nursery system. In the third set of crosses, fertilized eggs were again divided into diploid and triploid treatment groups, and the latter were treated with CB. At present, all of these families are growing well in our nursery system.

Conclusions: Causes of Pacific Oyster Mortality

Most of the Pacific oyster seed produced in commercial hatcheries never reaches the market because of deaths that occur during larval life, at the time of metamorphosis, and on the grow-out beds. The causes of these deaths are manifold, and this project has by no means solved the problem. We have, however, developed the facilities and are producing genetic groups and pedigreed stocks that will make possible rigorous scientific study of the causes of oyster mortality. Along the way, we have uncovered clues concerning specific causes and have opened up certain promising avenues for future research; these are briefly reviewed in summation to this report.

Patterns of larval mortality in our factorial mating experiments support Lannan's conclusion (1980) that larval mortality is contingent on genetic variation in the conditioning responses of broodstock. Gametes from certain parents appear to be incapable of larval development. This observation clearly needs to be pursued in a manner that will allow detailed physiological and biochemical characterization of gamete quality. Pedigreed and inbred stocks requisite for this work are being developed.

Even under optimal conditions, only about 30% of eyed-larvae successfully set (Broadley, 1986; Ken Cooper, Coast Oyster Co., personal communication). Again, the causes of mortality at this critical stage in development are many. There is good evidence that larval nutrition is important in determining successful metamorphosis (see Hedgecock, 1985a). A genotypic component of larval fitness has also been inferred from observations of widespread homozygote deficiencies in natural bivalve populations (Singh and Green, 1984); inbreeding studies have yielded mixed results, although there is slight evidence that homozygotes are at an advantage during the larval phase (Blanc and Bonhomme, 1986). In our current Sea Grant project, we have attempted experimentally to manipulate heterozygosity both upward and downward to test these hypotheses.

Our observation of a consistent positive relationship between the density of larval cultures and the time to metamorphosis is another important clue. By adjusting larval density after initial deaths, we can more accurately measure differences among families in later mortality, growth, and development and refine our understanding of their role in variation in set.

Summer mortality of oysters may be related to the metabolic stresses

of reproductive maturation (Perdue et al., 1981). Triploid oysters are thus expected to show greater resistance to summer mortality because of their greatly retarded sexual development (Downing and Allen, 1987). Grow-out of triploids with diploid controls will attempt to refine this prediction by sorting out the confounding effects of increased heterozygosity in triploids from the sterility of triploids. Nevertheless, to the extent that triploidy is a useful commercial production method, there is need to find alternatives to the effective but highly toxic drug, CB, that is now used to induce triploidy. Our experiments with the cytochalasin D show some promise that less toxic inducers may be found.

Finally, our discovery that commercial stocks of Pacific oysters are in danger of becoming inbred because of the use of small breeding populations should allow the oyster industry to avert declines in future performance of hatchery stocks. Inbreeding, which reduces heterozygosity, is expected also to reduce growth rate because of the positive relationship that has been shown to exist between heterozygosity and growth rate (Foltz et al., 1983; Singh and Green, 1984; Zouros and Foltz, 1984; Garton et al., 1984; Fujio, 1982). As already discussed, the effects of inbreeding on mortality are less easily forecast, but in general inbreeding can be expected to affect survival negatively. We have made our results known to commercial hatcheries and have discussed with them ways to improve broodstock management so as to ameliorate this problem.

Cooperating Organizations

Coast Oyster Company, Quilcene, Washington
Great American Shellfish Company, Marshall, California
Hog Island Shellfish Company, Marshall, California
Johnson's Oyster Company, Inverness, California
Tomales Bay Oyster Company, Marshall, California
University of California Extension, Davis, California
Western Regional Aquaculture Consortium

References

Blanc, F., and F. Bonhomme. 1987. Polymorphisme genetique des populations naturelles de mollusques d'interet aquicole. In *Selection, hybridization and genetic engineering in aquaculture.* K. Tiews, ed. Berlin, Germany.

Borgeson, W., Hedgecock, D., Nelson, K. and Sly, F. 1989. A novel, small-scale system for experimental culture of oyster and other aquatic larvae. Poster presented at meeting of World Aquaculture Society, Aquaculture-89, Los Angeles, California, January 1989.

Broadley, T. 1986. The effects of temperature, salinity and food when setting hatchery reared larvae of *Crassostrea gigas. B.C. Maric. Newslett.* 6(3):8–15.

Coon, S. L., D. B. Bonar, and R. M. Weiner. 1985. Induction of settlement and metamorphosis of the Pacific oyster, *Crassostrea gigas* (Thunberg), by L-dopa and catecholamines. *J. Exp. Mar. Biol. Ecol.* 94:211–221.

Chew, K. K. 1984. Recent advances in the cultivation of molluscs in the Pacific United States and Canada. *Aquaculture* 39:69–81.

Downing, S. L., and Allen. 1987. Induced Triploidy in the Pacific Oyster, *Crassostrea gigas*: Optimal treatments with cytochlosin B depend on temperature. *Aquaculture* 61:1–15.

Foltz, D. W., G. F. Newkirk, and E. Zouros. 1983. Genetics of growth rate in the American oyster: Absence of interactions among enzyme loci. *Aquaculture* 33:157–165.

Fujio, Y. 1982. A correlation of heterozygosity with growth rate in the Pacific oyster, *Crassostrea gigas. Tohuku J. Agric. Res.* 33(2):66–75.

Garton, D.W., R. K. Koehn, and T. M. Scott. 1984. Multiple-locus heterozygosity and the physiological energetics of growth in the coot clam, *Mulina lateralis.* from a natural population. *Genetics* 108:445–455.

Hedgecock, D. 1985a. Final report for UCSG Project R/NP-1-14B. California Sea Grant College Program, La Jolla, California.

Hedgecock, D. 1985b. Progress report for UCSG Project R/A-65. California Sea Grant College Program, La Jolla, California.

Hedgecock, D. 1986. Progress report for UCSG Project R/A-65. California Sea Grant College Program, La Jolla, California.

Hedgecock, D. 1987. Progress Report for UCSG Project R/A-65. California Sea Grant College Program, La Jolla, California.

Hershberger, W. K., J. A. Perdue, and J. H. Beattie. 1984. Genetic selection and systematic breeding in Pacific oyster culture. *Aquaculture* 39:237–247.

Lannan, J. E. 1980. Broodstock management of *Crassostrea gigas*: I. Genetic variation in survival in the larval rearing system. *Aquaculture* 21:323–336.

Lu, J.-K. 1986. The effects of temperature and salinity on meiosis, fertilization, and first mitosis of the Pacific oyster (*Crassostrea gigas*) eggs. Master's thesis, University of Washington, Seattle.

Perdue, J. A., J. H. Beattie, and K. K. Chew. 1981. Some relationships between gametogenic cycle and summer mortality in the Pacific oyster (*Crassostrea gigas*) in Washington state. *J. Shellfish Res.* 1:9–16.

Quillet, E., and P. J. Panelay. 1986. Triploidy induced by thermal shocks in Japanese oyster *Crassostrea gigas. Aquaculture* 57:271–279.

Singh, S. M., and R. H. Green. 1984. Excess of allozyme homozygosity in marine molluscs and its possible biological significance. *Malacologia* 24:569–581.

Zouros, E., and D. W. Foltz. 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia* 25:583–591.

Publications and Lectures

Borgeson, W., Hedgecock, D., Nelson, K. and Sly, F. 1989. A novel, small-scale system for experimental culture of oyster and other aquatic larvae. Poster presented at meeting of World Aquaculture Society, Aquaculture-89, Los Angeles, California, January 1989.

Hedgecock, D. 1987. Project progress report on R/A-65, Development of Pacific oyster broodstock. California Sea Grant College Program, La Jolla, California.

Hedgecock, D. 1988. Can the Pacific oyster be domesticated? In *West Coast Mollusc Culture: A Present and Future Perspective.* R. Amidei, ed. Report No. T-CSGCP-017. California Sea Grant College Program, La Jolla, California. pp. 69–72.

Hedgecock, D., and F. L. Sly. 1988. Genetic drift and effective population sizes in commercial stocks of the

Pacific oyster *Crassostrea gigas* on the U.S. west coast. *J. World Aquacult. Soc.* 20:184. Abstract.

Sly, F., and Hedgecock, D. 1988. Genetic drift and effective population sizes in commercial stocks of the Pacific oyster *Crassostrea gigas* on the U.S. west coast. Presented at the annual meeting of the National Marine Fisheries Association, Eastern Division, New Orleans, Louisiana, June 27, 1988.

Sly, F., and Hedgecock, D. 1988. Genetic drift and effective population sizes in commercial stocks of the Pacific oyster *Crassostrea gigas* on the U.S. west coast. Presented at the annual meeting of the National Marine Fisheries Association, Western Division, Olympia, Washington, September, 16, 1988.

Sly, F., and Hedgecock, D. 1989. Accepted. Genetic drift and effective population sizes of hatchery-propagated stocks of the Pacific oyster *Crassostrea gigas. Aquaculture.*

Fisheries

Quantitative Evaluation of *C. botulinum* Growth Risk in Seafood Stored at Low Temperatures under Modified Atmospheres

University of California, Davis
R/F-99
Project Initiated: October 1, 1984
Project Completed: September 30, 1987

Constantin Genigeorgis

In recent years, rapid expansion has occurred in the use of modified (vacuum, gas, hypobaric) atmospheres (MA) in the cold storage of foods of plant and animal origin to extend their shelf life and facilitate long distance shipping. The public health implications of the new technologies with respect to the potential growth of pathogenic microorganisms in foods stored under cold or abused temperature conditions has not been adequately evaluated. Nonproteolytic type B, E, and F strains of *Clostridium botulinum* are able to grow in temperatures as low as 3.3°C (38°F). Inhibition of growth of the normal microbial flora of food by MA may allow a selective growth of *C. botulinum* sufficient to be a health hazard of unknown risk. In seafood the probability of growth may be amplified by the high prevalence of *C. botulinum*. Presence of high numbers of *C. botulinum* spores in seafood may also require adjustment in further processing technology to ensure safety. The long-term objective of this study has been the quantitative evaluation of the effects of variables such as *C. botulinum* types, numbers, cell type, MA, temperature, time of storage, and chemical preservatives on the probability of *C. botulinum* growth in various species of fresh fish. From October 1, 1984 to September 30, 1987, a number of these aspects were explored; the most significant findings are reported here.

Probability of *C. botulinum* Types B, E, and F Growth in Raw Salmon.

Three factorial design inoculated-pack experiments were run. In experiment 1, fish muscle homogenates without skins were prepared aseptically from fresh whole gutted fish (3 to 4 days old) obtained from a wholesale store and frozen until used. Only fish containing no *C. botulinum* or a type other than the inoculum was used. Toxin produced by resident *C. botulinum* was neutralized in toxin assays. Defrosted fish homogenate (approximately 1.5 g/well) was added to 24-well tissue culture plates and then inoculated with 20 µl of spore suspension. Additional homogenate (1.5 g/well) was added to each well, and the plates were covered with lids and placed in 20 x 50 cm barrier bags (Cryovac type B540 with O_2 transmission of 30–50 ml/m^2/24 hr at 1 atm and 22.8°C). Modified atmospheres of vacuum, 100% CO_2, or 70% CO_2 + 30% air were created by using a Multivac A300 model 22 packaging machine. The experiments were arranged in a factorial design, including seven spore levels (10^4 to 10^{-2} spores/sample) of a pool of non-proteolytic spores (equal levels of 4B, 5E, and 4F) and control, triplicate inoculations, three MA, six temperatures of incubation (1, 4, 8, 12, 16, and 30°C), and multiple samplings of up to 60 days. At a particular day and for each temperature and MA condition, a set of 21 samples representing the seven inoculation levels plus control were removed for total plate counts (TPC), measurement of pH, gas analysis, toxicity testing, and estimation of the probability of toxigenesis. For toxicity the standard mouse test was used, and the samples were kept frozen until analysis. The lowest inoculum level was typed to verify that toxicity was due to the inoculum. Probability of growth and toxigenesis was calculated by a 7 x 3 MPN method.

In experiment 2, tissue homogenate was prepared aseptically from 4-hour-old migrating salmon (seven fish) and frozen until use. Samples were removed from the freezer and used for inoculated pack studies immediately after defrosting and after incubation at 4–8°C for 3 to 6 days to obtain three levels of initial microbial load. In this experiment, only the 70% CO_2 MA was used for storage at temperatures and times similar to experiment 1. Type and levels of spore inocula also remained the same.

In experiment 3, salmon tissue homogenates were replaced by salmon fillets. The fillets (25 g each) were cut and placed one on top of the other in 20 x 25 cm barrier bags. Next, 20 µl of each inoculum dilution was dispensed between the two fillets to accomplish spore levels of 10^4, 10^3, 10^2, 10^1, and 10^0 per set of fillets. Bags packed with fish under the three MA were incubated at 1, 4, 8, 12, and 20°C for up to 60 days. Fillet samples were assessed for odor and appearance by a panel of three persons in addition to other measurements described in experiment 1. The probability of toxigenesis increased with temperature and inoculum size. Analysis of variance showed significant effects due to MA; temperature (T); storage time (ST); and MA x T, MA x ST, and T x ST interactions at 4–16°C for up to 21 days. Toxin was first detected after 1 day at 30°C, 2 days at 16°C, 6 days at 12°C, and 9–12 days at 8°C. Toxin was detected in fish stored at 4°C for 15 days under vacuum but not in those stored under CO_2 for up to 60 days.

In experiment 2, analysis of variance showed the highly significant effect ($P < .005$) of the initial microbial flora (IMF), T, ST, IMF x T, and T x ST on the

probability of toxigenesis. A significant effect ($P < .05$) of MA, T, ST, and ST x MA on the probability of toxigenesis was shown for storage at 8–12°C for up to 18 days. At 8 and 12°C under vacuum, 100% CO_2, and 70% CO_2, toxigenesis was detected first after 6, 9, 12, 3, 6, and 6 days, respectively. No toxigenesis was observed at 4°C. At 30°C, toxigenesis coincided with spoilage. At 8 and 12°C, toxigenesis preceded spoilage. At 4°C, all fish spoiled at 24–48 days.

Mathematical formulas were derived that were predictive of the probability (P) of toxigenesis for a certain T, ST, IMF, and spore inoculum (I). Linear regression analysis was used for the data relating the log of the lag phase (LP) of toxigenesis to T, log I, and log IMF, and logistic regression was used for the data relating the P during the exponential phase of toxigenesis to T, ST, and LP. Combination of the two regression analyses gave the following predictive model formula for P:

$$Log_{10}\ (P\%) = 5(e^{Y}/1 + e^{Y}) - 3$$

where
$Y = a + b(T) + b_2(ST - LP) + b_3(ST - LP)(T)$; $\log_{10} LP = a + b_1(T) + b_2(1/T)b_3(log_{10} I) + b_4(log_{10} IMF)$; $a = intercept$; and b_1, b_2, b_3, and b_4 are regression coefficients. In all three experiments, excellent correlation was obtained between observed and predicted data.

Probability of Toxigenesis in Red Snapper Fillets

This experiment was similar to the salmon fillet experiment with storage of only up to 21 days. Toxin was detected after incubation of 1 day at 30°C, 9–12 days at 12°C, and 9–12 days at 8°C. No toxin was detected in any of the fillets stored at 4°C. On the basis of formulas developed for red snapper, correlation between observed and predicted probabilities was good.

Typing of the toxin produced in the experiments discussed indicated the presence of only type B toxin though types B, E, and F strains were inoculated into the fish samples. It was for this reason that following experiments were based on the use, as inoculum, of only spores of the same type.

Probability of *C. botulinum* Types E and F Growth in Raw Rock Fish Tissue Homogenate

The experiments were similar to the salmon experiments. Two MA (100% CO_2 and vacuum), separate E and F spore inocula (each made of four strains), and multiple sampling of up to 60 days were used.

Only type E spore inocula and not type F were able to grow and produce toxin. The F spore pool grew in brain heart infusion broth at 30 and 16°C within 6 and 9 days with 10 and 10^2 spores, respectively. Statistical analysis indicated that the length of the LP of toxigenesis was affected significantly by MA, T, I, T x I, and MA x T x I ($P < .001$ to $< .05$). The probability of toxin production by one type E spore was affected significantly by MA ($P < .05$) and by T and I ($P < .001$) and not significantly by MA x T, MA x I, and T x I ($P > .1$). At the 10^4 inoculum level, toxin was detected first in fish stored under vacuum after 2, 3, 6, 9, 21, and >60 days at 30, 20, 16, 12, 8, and 4°C, respectively.

The derived formula for LP and vacuum was $\log_{10}LP = 1.19 - 0.03(T) - 3.38(1/T) - 0.06(I)$, with $R^2 = 0.96$ and standard error (SE) = 0.113. For 100% CO_2, $\log_{10}LP = 1.48 - 0.05(T) - 2.46(1/T) - 0.06(I)$, with $r^2 = 0.95$ and SE = 0.13, and for the combined data of both MA, $\log_{10}LP = 1.34 - 0.04(T) + 0.82(1/T) - 0.06(I)$, with $R^2 = 0.945$ and SE = 0.13.

The formulas showed the good correlation between observed and predicted probabilities of toxigenesis. Comparison of the present findings with those previously found for rockfish indicated that the E pool strains grew at a slower rate than the B strains in the pool made of B, E, and F strains.

Probability of *C. botulinum* Type E Growth in Raw Salmon Tissue Homogenate

The experimental design was similar to previous ones with inoculated packs. Two MA, seven temperatures, seven levels of an E spore pool in triplicate, controls, and storage up to 60 days were used. Stepwise regression analysis yielded the following equation relating LP (in days) to MA, T, and I: $\log_{10}LP = 0.953 + 0.089(MA) - 0.043(T) + 4.523(1/T) - 0.082(I)$, with $R^2 = 0.962$. The value of MA is 0 for vacuum and 1 for 100% CO_2. In the model, T accounted for 85.3% of the variation, and 1/T, I, and MA for 5.8%, 4.6%, and 0.3%, respectively. Packaging in 100% CO_2 extended the LP by 1.2 days as compared with vacuum, an effect that may provide a slight increase in the safety when the products are exposed to short periods of temperature abuse. Increasing the spore load from one to 10^4 caused a decrease of 1.2–2.1 days in the predicted LP for a given storage temperature. Thus, any level of initial spore load may affect a product's integrity adversely during low storage temperature fluctuations. The developed models for the probability of toxigenesis by 1 spore were $P = e^{Y}/1+e^{Y}$, where for 100% CO_2, $Y = -4.97 + 0.04(T) - 0.09(ST - LP) + 0.03(T)(ST - LP)$, and for vacuum, $Y = -3.13 + 0.11(T) + 0.53(ST - LP) - 0.01(T)(ST - LP)$. No type E toxin was detected in fish stored at 4°C even in the presence of $10^{5.239}$ spores/sample of 3 g. The rate of change in probability increased more rapidly as the temperature of incubation approached the optimum for *C. botulinum* growth. The pH of stored fish remained at levels of <7.0 for vacuum storage and 6.2–6.3 for CO_2 storage. Type A spores were present in the raw fish, and the initial microbial flora of the salmon was <100/g.

Probability of *C. botulinum* Type E Growth in Raw Dover Sole Tissue Homogenate

The experimental design was

similar to that used in the previous experiment. Analysis of variance did not show any significant effect of MA (100% CO_2 vs. vacuum) on length of LP. The regression formula derived for the length of LP in days is $\log_{10}LP = 1.543 - 0.042(T) + 1.742(1/T) - 0.043(I)$, with $R^2 = 0.90$ and SE = 0.18. In the model $P = e^y/1 + e^y$ for 100% CO_2, $y = 10.017 + 0.193(T) - 10.825(ST - LP) + 1.376(ST - LP)T$, and for vacuum, $y = 6.825 - 0.004(T) - 7.370(ST - LP) + 1.538(ST - LP)T$.

Effect of Selected Chemicals on *C. botulinum* Type E Toxigenesis

Certain chemicals, used as fish dips to extend shelf life and minimize drip-loss, have been suggested as additional boosters to the safety of refrigerated fish stored under MA with respect to *C. botulinum*. In this experiment we compared the effect of such chemicals on the toxigenesis of type E *C. bolulinum* in fresh rockfish. Four fish muscle homogenates were prepared with added 0.25% potassium sorbate (K-S); 0.5% sodium tripolyphosphate (TPP); 0.25% K-S + 0.5% TPP; and 0.625% "Fish-Plus," a commercial product, respectively. Tissue homogenates in microsystem plates were inoculated in triplicate with seven levels (10^5–10^{-1}) of a *C. botulinum* type E spore pool composed of four strains at equal levels and controls. The plates were incubated under vacuum at 4, 8, 12, and 30°C for up to 45 days. The design included 140 combinations of T, chemicals, and sampling day, each combination made of 24 fish samples taken at various intervals and analyzed for toxin as done in the past.

The initial microbial flora of the fish was <100/g and the initial pH 6.6–6.7. No toxin was detected at 4°C during 45 days of storage. Analysis of variance of the probability of toxigenesis showed that ST, T, and ST x T were significant ($P < .0001$) factors able to explain the differences between chemical treatments; however, the chemical treatment itself was not significant ($P > .3$). At 8°C, a beneficial effect of chemicals in at least doubling the length of the LP was observed. When temperature was entered as a covariate, all chemical groups were deemed to be the same ($P > .1$) at 8, 12, and 16°C. TPP alone was slightly more conducive to *C. botulinum* growth at 30 C. Preliminary conclusions indicate that although sorbate may serve to preserve organoleptic qualities of fresh fish and may have some inhibitory effects on *C. botulinum* growth and toxigenesis, sorbate does not present an important "hurdle" to significantly decrease the probability of *C. botulinum* toxigenesis in MA-packaged fish. This conclusion is supported by the mode of action of sorbates in food systems, which is pH dependent with more antimicrobial effect at lower pH levels. The pH of fresh fish is near 6.7–6.8 and increases with age. Thus, for sorbates to exert their maximal effect, the pH of fresh fish would need to be lowered artificially.

Concluding Remarks

The overall objectives of this multiyear project were to develop a quantitative approach to assess the safety of new food processing technologies and their application on the question of *C. botulinum* toxin development in MA-packaged fish. To date, 927 individual experiments have contributed to a "general" linear regression formula for the prediction of the length of the LP in MA-packaged fish. Numerous variables were explored for their effect on LP. A significant effect on the length of LP was exhibited by fish type ($P < .001$), spore pool ($P < .001$), temperature ($P < .0$), 1/T ($P < .001$), spore inoculum ($P < .0$), MA ($P < .0205$), and initial aerobic plate count ($P < .0179$); the initial pH and the type of fish tissue (homogenate or fillet) were not significant at $P = .05$.

Developing a general predictive model entails consideration of the most botuligenic situations that may occur in a commercial environment so as to obtain a conservative estimate for storage safety. Thus, large inocula of psychrotrophic, nonproteolytic *C. botulinum* spores were inoculated into fish muscle where the Eh would be very low. Vacuum-packaged salmon inoculated with nonproteolytic *C. botulinum* type B, E, F spore pool yielded the following model capable of predicting the shortest time before toxigenesis: $\log LP = 1.03 - 0.04(T) + 2.74(1/T) - 0.09(I) - 0.04(IMF)$, with $R^2 = 0.89$ and S.E. = 0.22. According to this model, the storage time is approximately doubled when the initial spore inoculum is reduced from 10^4 to 10 spores/g, that is, from 5 to 10 days at 8°C. On the basis of this model, a comparison was made between data for toxigenesis reported in the literature and predicted LP for the same spore load and temperature of storage. This comparison showed the excellent ability of the model to predict the behavior of *C. botulinum* in fresh fish stored under MA.

Cooperating Organizations

Centers for Disease Control, Atlanta, Georgia

W. R. Grace Co., Cryovac Division

Publications

Baker, D. A., and C. Genigeorgis. In press. Predicting the safe storage of fresh fish under modified atmospheres, with respect to *Clostridium botulinum* toxigenesis by modeling length of the lag phase of growth. *J. Food Protection.*

Baker, D. A., C. Genigeorgis, J. Glover, and V. Razavilar. In press. Growth and toxigenesis of *C. botulinum* type E in fishes packaged under modified atmospheres. *Intl. J. Food Safety.*

Baker, D. A., C. Genigeorgis, and G. Garcia. Submitted. Prevalence of *Clostridium botulinum* in seafood and significance of multiple incubation temperatures for determination of its presence and type in fresh retail fish.

Garcia, G., and C. Genigeorgis. 1986. Quantitative evaluation of *Clostridium botulinum* non-proteolytic types B, E and F growth risk in fresh salmon tissue homogenates stored under modified atmospheres. *J. Food Prot.* 50:390–397.

Garcia, C., C. Genigeorgis, and S. Lindroth. 1986. Risk of *Clostridium botulinum* non-proteolytic types B, E and F growth and toxin production in salmon fillets stored under modified

atmospheres at low and abused temperatures. *J. Food Prot.* 50:330–336.

Genigeorgis, C. 1985. Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish: A review. *Intl. J. Food Microbiol.* 1:237–251.

Genigeorgis, C. 1986. Assessment of the risk of growth of *C. botulinum* non-proteolytic types B, E, and F in fresh fish stored at low temperatures under modified atmospheres. Presented at the 14th Congress of Microbiology, Manchester, England. Abstract.

Genigeorgis, C. A., J. Meng, and D. A. Baker. Submitted. Behavior of nonproteolytic *Clostridium botulinum* type B and E spores in cooked turkey rolls as affected by inoculum, sodium chloride concentration, temperature and time of storage. *J. Food Sci.*

Genigeorgis, C., and S. Lindroth. 1985. Potential *C. botulinum* risks in muscle foods stored under modified atmospheres. *Proc. 9th Intl. Symp. World Assoc. Vet. Food Hyg.* 2:200–206.

Ikawa, J. 1986. Effect of temperature and storage time on the probability of growth of non-proteolytic *Clostridium botulinum* types, B, E and F spore pools in a model broth and in rock fish fillets. Master's thesis, University of California, Davis.

Ikawa, J., and C. Genigeorgis. 1986. Probability of growth and toxin production by non-proteolytic *Clostridium botulinum* in rock fish fillets stored under modified atmospheres. *Intl. J. Food Microbiol.* 4:167–181.

Ikawa, J., C. Genigeorgis, and S. Lindroth. 1986. Temperature and time effect on the probability of *Clostridium botulinum* growth in a model broth. *Proc. 2nd World Congr. Food borne Infections Intoxications. 1:370–374.*

Jensen, M. J., Genigeorgis, and S. Lindroth. 1986. Probability of growth of Clostridium botulinum at various temperatures in a model broth system. *J. Food Saf.* 8:109–126.

Lindroth, S., and C. Genigeorgis. 1986. Probability of growth and toxin production by non-proteolytic *Clostridium botulinum* in rock fish stored under modified atmospheres. *Intl. J. Food Microbiol.* 3:167–181.

Lectures

Genigeorgis, C. Invited speaker, Western Food Industry Conference, Davis, California, 1985.

Genigeorgis, C. 9th International Symposium World Assoc. Veterinarian Food Hyg., Budapest, Hungary, 1985.

Genigeorgis, C. Invited speaker, Institute of Food Technologists annual meeting, Atlanta, Georgia, 1985.

Genigeorgis, C. Invited lecture, Greek Institute of Food Technologists, Athens, Greece, 1985.

Genigeorgis, C. Invited lecture, College of Agriculture, Athens, Greece, 1985.

Genigeorgis, C. Invited seminar, Dept. of Food Science, University of Calironia, Davis, 1985.

Genigeorgis, C. Panamerican Health Organization Consultant, Food Safety and Processing, postgraduate course, National Autonomous University of Mexico City, 1985 and 1986.

Genigeorgis, C. Invited by the University of Helsinki and the Finnish Society of Food Science and Technology for a series of lectures on modified atmospheres and water activity, Helsinki, Finland, 1986.

Genigeorgis, C. Invited speaker, 37th annual meeting, Pacific Fisheries Technologists, Vancouver, Canada, 1986.

Genigeorgis, C. Speaker, 14th International Congress of Microbiology, Manchester, England, 1986.

Genigeorgis, C. Invited for a series of lectures on food safety and preservation at the U.S. Airforce, School of Aerospace Medicine, San Antonio, Texas, 1986.

Baker, D., and C. Genigeorgis. 38th annual meeting, Pacific Fisheries Technologists, Monterey, California, 1987.

Baker, D., and G. Genigerogis. The role of quantitative risk assessment in the development and safe commercialization of refrigerated, modified atmosphere packaged foods. Inter-Agency Botulism Research Coordinating Committee, Madison, Wisconsin, 1987.

Genigeorgis, C. Invited for a series of lectures on processing and safety by the Aerospace School of Medicine, San Antonio, Texas, 1987.

Genigeorgis, C. Invited speaker for a series of lectures on the safety of processed foods by the Academy of Preventive Medicine, Salt lake City, Utah, 1987.

Genigeorgis, C. Invited speaker, 13th International Symposium on Toxins in Foodborne Disease, Halkidiki, Greece, 1987.

Genigeorgis, C. Invited speaker, Gorman Conference on packaging, Lake Geneva, Illinois, 1987.

Kidney Diseases of Pacific Salmon

University of California, Davis
R/F-100
Project Initiated: October 1, 1984
Project Completed: September 30, 1987

Ronald P. Hedrick

The overall objective of this project is to determine the source, mode of transmission, and effect of proliferative kidney disease (PKD) on salmonid fish. During the project, considerably more has been learned about the nature of PKX, the parasite that causes PKD, and its effects on salmon and trout.

Characteristics of the Disease and Pathogen

A sequential examination of the disease from the earliest known stages to resolution was conducted to determine different developmental forms of the PKX parasite and their effects on the host (Kent and Hedrick, 1986a). These studies indicated that the parasite is first recognized approximately 3–4 weeks after fish are exposed to water known to be enzootic for the disease (at water temperatures of 15–17°C). Vegetative development of these early stages (trophozoites) results in a large increase in the number of parasites in the vascular system, and they are particularly evident in the larger sinuses of the kidney (Kent and Hedrick, 1986a). At this stage, the parasite can be easily transmitted to uninfected salmon or trout by intraperitoneal injection of infected kidney tissues.

During the vegetative development, the host response can be intense, and the influx and proliferation of the host's macrophages and lymphocytes result in a diffuse granulomatous lesion (Ferguson and Needham, 1979). Because the primary site for parasite development is the kidney, this response manifests itself as the gross swelling typical of the disease. Kent and Hedrick (1986b) showed that the host's inflammatory response could be greatly diminished by administering cortisol implants to PKD affected fish. Although this method is not practical for the treatment of the disease, it did show that the inflammatory response can influence the development of PKX. In fish receiving cortisol treatment, the parasite rapidly passed from the vegetative stages in the vascular system and the kidney interstitium through the epithelium of the kidney tubules and into the lumen. At this site numerous early sporogonic stages of the parasite were detected as compared with fish with PKD not treated with cortisol (Kent and Hedrick, 1986b).

The next developmental stages of PKX to be observed are early spore stages in the lumen of the kidney tubules (Kent and Hedrick, 1986a). These stages, although usually not abundant, are prominent only in the later stages of the disease after the kidney swelling and interstitial forms of PKX have diminished (approximately 9 weeks after exposure at water temperatures of 15–17°C). The spores formed at this stage of the disease show formation of polar capsules, an important characteristic of the phylum Myxozoa. However, spore development is incomplete as no valves protecting the internal or sporoplasm cells arise. This incomplete development of the spore explains the difficulty experienced by earlier workers trying to establish the taxonomic placement of the parasite; these stages are rarer, although present, among fish in European outbreaks of PKD. The incomplete development of the spore and the inflammation associated with the presporogonic stages of PKX in the salmonid (both atypical for most myxozoan parasites) may also indicate that the parasite is infecting an atypical host. On the basis of the characteristics of the immature spores seen in salmonid fish, Kent and Hedrick (1986a) hypothesized that the mature spore would resemble those known for the genus *Sphaerospora*. These parasites are commonly found in the kidney of many cyprinid fishes and usually are nonpathogenic (Lom et al., 1985).

Determination of the Peak Periods of the Infectious Stage of PKX

A thorough examination of the seasonality of the infective stage was conducted at the American River Hatchery, a location with annually recurring outbreaks of PKD in their salmonid populations (Foott and Hedrick, 1987). This study indicated that the infectious stage was present in the hatchery water supply from April through October. These months corresponded to peak water temperatures, and this may be the environmental cue that causes this unknown stage to become infectious. A knowledge of the presence or absence of the infectious stage in the water at the hatchery has been used by management (California Department of Fish and Game) to predict when fish should be exposed to the parasite in order to later obtain fish immune to the disease. The latter fish are allowed to be planted for recreational fisheries, whereas movement of the former is restricted.

Potential Hosts and Reservoirs of the PKX Parasite

The parasite (PKX) causing PKD cannot be transmitted from one salmonid fish to the other by any natural routes (cohabitation of infected fish with uninfected fish or feeding of infected fish tissues to uninfected fish). The only successful routes of transmission of PKX have been exposure to water containing the infectious stage or direct injection of tissues from an infected fish into a healthy recipient.

This indicates that another reservoir host is the likely source of infectivity. In this regard we have examined other species (nonsalmonid) of fish as possible sources of the parasite. This hypothesis is supported by epizootiological studies and by structural similarities between PKX and the parasites found among certain nonsalmonid hosts. A parasite remarkably similar to PKX has been found in cyprinid fish inhabiting the water supply of a hatchery with recurrent PKD episodes (Hedrick et al., 1987). Further studies on the relationship of this parasite to PKX are underway.

Prevalence of the Parasite in Adult Salmon

As shown for other microbial diseases (Fryer and Sanders, 1981), a potential reservoir of the parasite might be infected adult salmon returning to the hatchery. An examination of 60 chinook salmon and 30 steelhead adults from Nimbus Hatchery and 30 steelhead adults and 30 chinook salmon adults from Mad River Hatchery showed no evidence of the PKX parasite or of proliferative kidney disease. These results indicated that yearly epizootics in the juvenile salmon are probably not a result of infections from adult salmon.

Preparation of Anti-PKX Antibodies

Because of difficulties in purifying the parasite from host kidney tissues no antibody has been produced. Repeated attempts to produce the antibody in rabbits by injections of heavily infected fish kidney materials have failed. Attempts to purify and use convalescent antibody from infected trout have also been unsuccessful. We are presently trying to produce parasite-specific monoclonal antibodies.

Susceptibility of Selected Pacific Salmon to PKD

A comparison of the development of PKX among juvenile chinook (*Oncorhynchus tshawytscha*), coho (*Oncorhynchus kisutch*), and sockeye (*Oncorhynchus nerka*) was made after experimental infection. All three species were susceptible to PKX. The chinook and sockeye showed considerably more morbidity and mortality. By 7 weeks after injection, all fish examined of these two species had full clinical signs of PKD. Several sockeye salmon died during the course of the study because of heavy PKX infections. The parasites were extremely abundant in the vascular system of the kidney where thrombi composed of PKX cells occluded many of the major vessels. At 13 weeks after injection, few interstitial and no vascular forms of PKX remained, but numerous developmental stages in the lumens of the kidney tubules were observed. Certain of these stages contained two polar capsules typical of later stages of the PKX myxosporean as observed in rainbow trout (*Salmo gairdneri*).

Determination of the Physical Properties of the Infectious Stage

The only property of the parasite that was examined in detail was the rapidity with which it could infect the salmonid host. Exposure periods for as short as 3 days were sufficient to infect rainbow trout held in water at American River Hatchery. Longer periods (7 days, 2 and 3 weeks) resulted in progressively increasing levels of infection. A period of 24 hours was not sufficient to infect fish in this particular study.

Possible Modes of Transmission of the Parasite

Several possible modes of transmission of the parasite were examined under laboratory conditions. Cohabitation of infected fish with uninfected fish failed to transmit the disease, as did feeding infected kidney tissues to healthy salmon. The only successful routes of infection were injections of infected kidney or spleen tissues or blood into healthy animals (Kent and Hedrick, 1985). The recipients showed all of the typical stages of the parasite and the disease in the same manner as fish exposed to water containing the infectious stage (the only other known method of transmitting the disease).

Effects of Water Temperature on the Progress of PKD

Rainbow trout with early infections with PKD were divided into replicate aquaria supplied with water at 10, 15, or 20°C. The progress of development of PKD was followed by weekly sampling of the fish. At 20°C, the disease was accelerated, and by 2 weeks at that temperature, the kidney swelling was down, the parasites had left the interstitium, and a few sporulating forms were found in the lumens of the tubules. The numbers of these forms steadily declined in the remaining weeks. At 15°C, the normal progress of the disease continued for an additional 3 weeks, with pronounced renal swelling and high numbers of parasites. Sporulating stages were abundant at 3 weeks and continued to be prevalent throughout the remainder of the study. At 10°C, the development was slowed, and the infection persisted (both renal swelling and numbers of parasites) for an additional 3 weeks past those in the 15°C group.

Effects of Seawater on the Progress of PKD in Pacific Salmon

Many salmon, particularly chinook salmon, are released each year from hatcheries in California. Because PKD impairs renal functions and causes a chronic anemia, its effect on out-migrating salmon was important to determine. The effects of transfer to seawater on the progress of PKD in chinook salmon juveniles with naturally acquired PKX infections therefore were examined. Clinical disease and PKX cells were observed in parallel groups of salmon held in 13°C seawater (33 ppt) or 13°C fresh water for up to 3 months. Development of the parasite and disease were similar, and later developmental stages, including immature spores, were observed in groups of salmon held in fresh and seawater.

Cooperating Organizations

California Department of Fish and Game
Ministry of Agriculture, Fisheries, and Food, Fish Disease Laboratory, United Kingdom
U.S. Fish and Wildlife Service

References

Ferguson, H. W., and E. A. Needham. 1978. Proliferative kidney disease in rainbow trout *Salmo gairdneri* Richardson. *J. Fish Dis.* 1:91–108.

Foott, J. S., and R. P. Hedrick. 1987. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson to re-infection. *J. Fish Biol.* 30:477–483.

Fryer, J. L., and J. E. Sanders. 1981. Bacterial kidney disease of salmonid fish. *Ann. Rev. Microbiol.* 35:273–298.

Hedrick, R. P., M. L. Kent, R. J. Toth, and J. K. Morrison. 1987. Fish infected with *Sphaerospora* spp. Thelohan (Myxosporea) from waters enzootic for proliferative kidney disease of salmonids. *J. Protozool.* 35:13–18.

Kent, M. L., and R. P. Hedrick. 1986a. The development of the PKX myxosporean in rainbow trout, *Salmo gairdneri* Richardson. *Dis. Aquat. Org.* 1:219–225.

Kent, M. L., and R. P. Hedrick. 1986b. Effects of cortisol implants on the development of PKX and associated inflammatory response in rainbow trout *Salmo gairdneri* Richardson. *J. Parasitol.* 73:455–461.

Lom, J., M. Pavlaskaova, and I. Dykova. 1985. Notes on kidney-infecting species of the genus *Sphaerospora* Thelohan (Myxosporea), including a new species *S. gobionis* sp. nov., and on myxosporean life cycle stages in the blood of some freshwater fish. *J. Fish Dis.* 8:221–232.

Publications

Foott, J. S., and R. P. Hedrick. 1986. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout *Salmo gairdneri* Richardson to re-infection. *Bull. Eur. Assoc. Fish Pathol.* 6:9–11.

Foott, J. S., and R. P. Hedrick. 1987. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout *Salmo gairdneri* Richardson to re-infection. *J. Fish Biol.* 30:477–483.

Hedrick, R. P. 1984. Proliferative kidney disease (PKD) of Pacific salmon. *Calif. Aquacult. Newslett.*, December.

Hedrick, R. P., and D. Aronstein. 1986. Effects of seawater on the progress of proliferative kidney disease (PKD) in chinook salmon (*Oncorhynchus tshawytscha*). *Fish Health Sect. Newslett.* 14:6.

Hedrick, R. P., M. L. Kent, and C. E. Smith. 1986. PKD, poorly understood protozoan to blame. *Salmonid* 10:9–12.

Hedrick, R. P., M. L. Kent, and C. E. Smith. 1986. Proliferative kidney disease among salmonid fishes. Fish Disease Leaflet. United States Department of Interior, Fish and Wildlife Service, Washington, D.C.

Hedrick, R. P., M. L. Kent, and R. J. Toth. 1986. Myxosporeans detected in non-salmonid fishes from waters enzootic for proliferative kidney disease. *Bull. Eur. Assoc. Fish Pathol.* 6:32–35.

Hedrick, R. P., M. L. Kent, and R. J. Toth. 1986. Myxosporeans detected in non-salmonid fishes from waters enzootic for proliferative kidney disease. *Fish Health Sect. Newslett.* 14:4.

Hedrick, R. P., M. L. Kent, R. Rosemark, and D. Manzer. 1984. Occurrence of proliferative kidney disease (PKD) among Pacific salmon and steelhead trout. *Bull. Eur. Assoc. Fish Pathol.* 3:34–37.

Hedrick, R. P., M. L. Kent, R. Rosemark, and D. Manzer. 1984. Proliferative kidney disease of Pacific salmon. *J. World Maric. Soc.* 15:318–325.

Hedrick, R. P., M. L. Kent, R. J. Toth, and J. K. Morrison. 1987. Fish infected with *Sphaerospora* spp. Thelohan (Myxosporea) from waters enzootic for proliferative kidney disease of salmonids. *J. Protozool.* 35:13–18.

Hedrick, R. P., M. L. Kent, J. S. Foott, R. Rosemark, and D. Manzer. 1985. Proliferative kidney disease (PKD) among salmonid fish in California: A second look. *Bull. Eur. Assoc. Fish Pathol.* 5:39–42.

Hedrick, R. P., M. L. Kent, J. S. Foott, R. Rosemark, and D. Manzer. 1985. Proliferative kidney disease (PKD) among salmonid fish in California: A second look. *Fish Health Sect. Newslett.* 13:4.

Hedrick, R. P., M. L. Kent, J. S. Foott, R. Rosemark, and D. Manzer. 1986. Proliferative kidney disease (PKD) among salmonid fish in California. In *Proliferative Kidney Disease in North America.* California Sea Grant, April 25–26, 1985, Davis, California.

Kent, M. L. 1985. The development of the PKX myxosporean, the causative agent of proliferative kidney disease, in rainbow trout *Salmo gairdneri* Richardson. Doctoral dissertation, University of California, Davis.

Kent, M. L., and R. P. Hedrick. 1985. PKX, the causative agent of proliferative kidney disease (PKD) of salmonid fish and its affinities with the Myxozoa. *J. Protozool.* 32:254–260.

Kent, M. L., and R. P. Hedrick. 1985. Transmission of the causative agent of proliferative kidney disease (PKD) with the blood and spleen of infected fish: Further evidence that the PKX parasite belongs to the phylum Myxozoa. *Bull. Eur. Assoc. Fish Pathol.* 5:36–38.

Kent, M. L., and R. P. Hedrick. 1985. Transmission of the causative agent of proliferative kidney disease (PKD) with the blood and spleen of infected fish: Further evidence that the PKX parasite belongs to the phylum Myxozoa. *Fish Health Sect. Newslett.* 13:4–5.

Kent, M. L., and R. P. Hedrick. 1986. Affinities of PKX with the phylum Myxozoa. In *Proliferative Kidney Disease in North America.* California Sea Grant, April 25–26, 1985, Davis, California.

Kent, M. L., and R. P. Hedrick. 1986. Effects of cortisol implants on the development of PKX and associated inflammatory response in rainbow trout *Salmo gairdneri* Richardson. *J. Parasitol.* 73:455–461.

Lectures and Conferences

Hedrick, R. P. Current diseases affecting aquaculture of finfish in California, USA. Seminar, Department of Zoology, National Taiwan University, Taipei, Taiwan, December 14, 1984.

Hedrick, R. P. Proliferative kidney disease in California, USA. Presented at the Proliferative Kidney Disease Workshop. Davis, California, April 25–26, 1985.

Hedrick, R. P. Affinities of PKX, the causative agent of proliferative kidney disease (PKD), to the phylum Myxozoa. Presented at the annual meeting of the European Association of Fish Pathologists, Montpellier, France, September 2–5, 1985.

Hedrick, R. P. Proliferative kidney disease (PKD) of salmonid fish: Current status and research at the University of California, Davis. Invited paper, United States Trout Farmers Association, Sun Valley, Idaho,

October 11, 1985.

Hedrick, R. P. Current status of proliferative kidney disease (PKD) in California. Presented at the 36th annual Northwest Fish Cultural Conference, Tacoma, Washington, December 3–5, 1985.

Hedrick, R. P. Certification, transplantation and catastrophic diseases: Their role in the success of aquaculture. Co-chairman and invited speaker, World Aquaculture Society, Reno, Nevada, January 19–23, 1986.

Hedrick, R. P. Proliferative kidney disease among salmonid fish in California. Invited speaker, Proliferative Kidney Disease Workshop, Sequim, Washington, June 23–24, 1986.

Hedrick, R. P. Myxosporeans in fish from waters enzootic for proliferative kidney disease. Presented at national meeting of American Fisheries Society, Fish Health Section, Martinsburg, West Virginia, July 21–24, 1986.

Hedrick, R. P. Present status of proliferative kidney disease of salmonid fish. Presented at national meeting of American Fisheries Society, Fish Health Section, Martinsburg, West Virginia, July 21–24, 1986.

Hedrick, R. P. Proliferative kidney disease (PKD) of salmonid fish. Presented at Ichthyopathology in Aquaculture, Dubrovnik, Yugoslavia, October 21–24, 1986.

Hedrick, R. P. Fish diseases: Update and prognosis. Invited speaker, Inland Fisheries Division Training Conference, California Department of Fish and Game, California Maritime Academy, Vallejo, California, March 16–19, 1986.

Hedrick, R. P. Current disease affecting cultured fishes in California. Invited paper, California Aquaculture Association, Long Beach, California. February 17, 1987.

Hedrick, R. P. Proliferative kidney disease of salmonid fish. Invited speaker, Mandelli Trout Farm, Northern Italy Trout Growers, Pavia, Italy, August 24, 1987.

Hedrick, R. P. Prinicpal diseases of cultured fishes in California, U.S.A. Seminar, National Veterinary Institute, Uppsala, Sweden, August 27, 1987.

Hedrick, R. P. Effects of PKX, the causative agent of proliferative kidney disease on three species of salmonid fish. Presented at the 3rd International Conference of the European Association of Fish Pathologists, Bergen, Norway, August 31–September 3, 1987.

Hedrick, R. P. Infectious hematopoietic necrosis virus (IHNV): Appearance in a new species. Presented at the 3rd International Conference of the European Association of Fish Pathologists, Bergen, Norway, August 31–September 3, 1987.

Hedrick, R. P. Myxosporean diseases of salmonid fish in California. Seminar, Fish Disease Laboratory, Faculty of Agriculture, Kochi University, Kochi, Japan, October 16, 1987.

Endocrine Control of Salmonid Development and Seawater Adaptation

University of California, Berkeley
R/F-101
Project Initiated: October 1, 1984
Project Completed: September 30, 1987

Howard A. Bern, Charles S. Nicoll, Richard S. Nishioka, and Graham Young

In 1986–1987, we continued several hatchery-based studies involving the thyroid hormone peak, optimal release times and effects of hatchery rearing, and the parr-smolt transformation, all in cooperation with hatchery and management personnel of the California Department of Fish and Game. At the same time, several lines of inquiry into hormones and seawater adaptation were pursued, including further studies on stunting in coho salmon.

We have previously observed distinct differences in thyroid hormone cycles in coho raised at Mad River and Iron Gate hatcheries. The Iron Gate population frequently shows erratic and possibly asynchronous plasma thyroxine cycles as compared with the well-defined and synchronized thyroxine elevation exhibited by the Mad River population. We have suggested that rearing conditions at Iron Gate, which is supplied with reservoir water that is relatively unchanging in physicochemical properties, may be too uniform; several lines of evidence suggest that minor changes in rearing conditions result in increased thyroidal responses during smoltification of coho. Because stock differences could also account for discrepancies in the thyroxine cycle between Iron Gate and Mad River hatcheries, we have begun a study in which development of Mad River coho stock is compared with two groups: Iron Gate coho raised either at Iron Gate or at Mad River. Sampling, including collection of data for use in morphometric analysis, is now complete. Blood levels of thyroid hormones, growth hormone, and prolactin will be measured.

Studies on water quality changes on thyroid hormone cycles in chinook salmon *(Oncorhynchus tschawytscha)* continued, using underyearlings. Fish were exposed briefly to about 5 parts per thousand (ppt) salt each week from May until August; plasma samples were collected 1 day after exposure to salt. In contrast to previous studies, the salt-treated group did not show a new moon–associated elevation in plasma thyroxine experienced in August by the control group; instead, it displayed a gradual thyroxine rise through July. However, the salt-treated fish were in better condition compared with control and production fish and showed no mortality.

Monitoring of thyroid hormone cycles in chinook and coho continued at several hatcheries as part of our continuing commitment to the California Department of Fish and Games's smolt-quality committee. In an ongoing attempt to ascertain the timing of smoltification and to determine the ideal time for release of chinook salmon from Merced Hatchery, plasma samples of underyearling fish were collected from May through August. Optimal release time is critical because extensive water diversions have reduced river flows in the San Joaquin system and consequently caused water temperature to rise far above levels ideal for migrating salmonids. In addition, if timing of releases and migration downstream can be predicted in advance, additional water release can be negotiated, and the gigantic pumps of the California Aqueduct at Tracy (which can reverse the seaward flow and consequently can misguide the downstream migrating salmon back upstream into the pumps) may be turned down or shut off during this critical period. In 1986, a significant thyroxine peak occurred at the time of the new moon, June 7. Presumably, if chinook salmon follow the pattern observed in coho, release at the peak of T_4 levels should result in optimal downstream migration, ocean survival, and adult returns. Additional information on thyroxine over several years and information on migratory activity after release must be gathered and analyzed before a meaningful conclusion can be obtained.

We undertook a large-scale study in 1986, in which we aimed to construct a relatively complete endocrinological and physiological profile of the parr-smolt transformation by correlating hormonal changes with changes in hypo-osmoregulatory ability and calcium and magnesium levels. Particular emphasis was placed on the relationship of changes in prolactin, growth hormone, and calcitonin with other changes. Patterns of thyroid hormones and cortisol were similar to those observed in previous years: Plasma levels of thyroid hormone showed a major increase in April, whereas cortisol levels, which began increasing in March, did not reach maximal levels until early June. Seawater adaptability was greatest during April and May, and a group of fish was transferred into seawater in mid-April, at a time when gill Na^+,K^+-ATPase levels were high. The transfer did not affect plasma thyroid hormone levels but, as anticipated, plasma cortisol levels were elevated in the first few days after transfer. Plasma prolactin levels increased in March but fell just before peak thyroid hormone levels. Prolactin levels dropped considerably after transfer of fish into seawater and remained low for the remainder of the sampling period. No well-defined changes

occurred in plasma levels of calcitonin during development, or after transfer of fish into seawater, despite changes in plasma calcium levels, again suggesting that calcitonin may not be a calcium-regulating hormone in salmon. Levels of growth hormone have not yet been measured. Inasmuch as prolactin displays changes relatively early in smoltification before peak thyroid hormone levels are achieved, this hormone may have more profound consequences on the smoltification process than originally anticipated.

Coho salmon display retarded growth (stunting) after premature transfer to seawater. Stunts are generally panhypoendocrine with the notable exception of their growth hormone levels, which are fourfold higher than those of their normally growing counterparts. The failure of stunts to grow in the presence of high circulating levels of growth hormone suggests that the growth deficiency may be primarily due to the decreased ability of stunt tissues to bind growth hormone, as reported previously. In the case of the liver, this would presumably result in decreased production of somatomedin. Previous studies suggested that normal growth resumes after transfer of stunts into fresh water. In order to study the relationship between external salinity and growth hormone levels further, groups of stunts and normally growing smolts were transferred into fresh water for 3 weeks. Although no significant changes were observed in body weight, the freshwater stunts were obviously in much better condition than their seawater controls. At the end of the acclimation period, plasma samples were obtained from seawater stunts and smolts, from seawater stunts and smolts that had been acclimated to fresh water, and from smolts that had been retained in fresh water past the time they would normally migrate to the ocean. Levels of plasma growth hormone in seawater stunts were again much higher than those in fish growing normally. However, levels of growth hormone in stunts acclimated to fresh water for 3 weeks were similar to those in normally growing coho. Levels of plasma prolactin in freshwater fish were higher than those in seawater fish, and there was no notable difference between prolactin levels in stunts and smolts. Interrenal tissue was taken from all groups to assess the competence of the tissue to respond to adrenocorticotropic hormone (ACTH), cyclic adenosine monophosphate, and pregnenolone, but data collection is not yet complete. Thyroid hormones will also be analyzed. The results so far obtained from this study suggest that further information on the ontogeny of the stunting phenomenon may be gained by studying the endocrine changes that occur as normal growth resumes after return of stunts into fresh water.

A phenomenon of stunted growth in Atlantic salmon was observed in Iceland in November 1986. Large numbers of stunted Atlantic salmon appeared during sea-pen rearing on the southwest coast of Iceland. It was found that levels of growth hormone were elevated to a similar extent as in coho stunts. It was concluded that a similar developmental disorder can occur in both *Salmo* and *Oncorhynchus* species after premature transfer to seawater.

In order to investigate the hormonal control of gill sodium, potassium-adenosine triphosphatase (Na^+,K^+-ATPase, the sodium pump) in coho salmon, a technique for the organ culture of gill filaments for up to 7 days was developed. Single primary filaments were cultured in minimum essential medium (MEM) with Hanks' salts supplemented with 0.3% bovine serum albumin and 25 mM HEPES buffer. During a 7-day period, exclusion of trypan blue was greater than 99%, and histological inspection indicated that structural integrity was maintained with slight diminution of the secondary lamellae. Intracellular concentrations of Na^+ and K^+ through 4 days of culture were unchanged from initial levels. Na^+,K^+-ATPase activity of gill tissue from intact salmon decreased by 70% after 4 days (from 4.3 to 1.4 µmol adenosine diphosphate [ADP]/mg protein/hr), whereas Na^+,K^+-ATPase activity of gill tissue from hypophysectomized salmon decreased by only 22% (from 1.0 to 0.8 µmol ADP/mg protein/hr).

Salmon growth hormone (0.01, 0.1, and 1.0 µg/ml) and triiodothyronine (0.01, 0.1, and 1.0 ug/ml) had no effect on Na^+,K^+-ATPase activity of gill tissue from freshwater-adapted yearling coho salmon. Cortisol (0.1, 1.0, and 10./0 µg/ml), however, significantly increased gill Na^+,K^+-ATPase activity over control levels in a dose-dependent manner after 2 days in culture. The relative ability of steroids to elicit a response was dexamethasone > cortisol ≥ 11-deoxycortisol > cortisone. Insulin (0.1, 1.0, and 10.0 µg/ml) had no effect on the *in vitro* induction of gill Na^+,K^+-ATPase activity by cortisol. In gill tissue from hypophysectomized coho salmon, growth hormone and cortisol each increased gill Na^+,K^+-ATPase activity, and in combination acted synergistically. This synergy suggests that the hormones may be acting by different mechanisms, which remain to be elucidated.

We hypothesized that the large increase in gill Na^+,K^+-ATPase activity that occurs in the spring during the parr-smolt transformation may be due to an increase in gill responsiveness to cortisol or growth hormone. Gill tissue from intact juvenile coho salmon was tested monthly (February to July) for its response to cortisol (1.0 and 10.0 µg/ml), salmon growth hormone (1.0 µg/ml), and both hormones together (10.0 and 1.0 µg/ml, respectively). In each experiment, gill Na^+,K^+-ATPase activity increased between 30% and 45% after 4 days of exposure to cortisol at 10 µg/ml, whereas salmon growth hormone had no significant effect either alone or in combination with cortisol. The results suggest that although gill

Na^+,K^+-ATPase activity is consistently responsive to cortisol, no major changes in tissue responsiveness to this hormone occur during the parr-smolt transformation.

Experiments over several years have shown that both thyroid hormones and growth hormone exert regulatory effects on the salmon interrenal tissue, but evidence to date suggests that only growth hormone has direct effects. In order to avoid the possible confounding influence of developmental changes on interrenal responsiveness to growth hormone, studies were undertaken to assess the potential usefulness of the interrenal tissues from hypophysectomized coho salmon for studies of this kind. Two-year old coho salmon were hypophysectomized and were placed in isotonic seawater for 10 weeks before experimentation. Groups of hypophysectomized fish received injections of triiodothyronine (1 μg/g body weight), growth hormone (5 μg/g body weight), or vehicle every other day for a total of three injections. Sham-operated fish were also injected with vehicle. Animals were sacrificed 1 day after the final injection, and interrenal tissue was prepared for *in vitro* incubation. Tissue was incubated with ACTH or pregnenolone for 3 hours, and media were analyzed for cortisol. Although there was a significant reduction in the responsiveness of tissue of hypophysectomized fish to ACTH, the magnitude of the change was less than anticipated on the basis of the mammalian literature, and growth hormone and triiodothyronine did not significantly enhance the response. Cortisol production by tissue of hypophysectomized fish incubated with pregnenolone was higher than by tissue from intact animals: Growth hormone and triiodothyronine both greatly enhanced cortisol production in the presence of pregnenolone. These results indicate that ACTH is not mandatory for the maintenance of interrenal responsiveness and again emphasize that growth hormone and thyroid hormones exert regulatory effects on this tissue.

Our Sea Grant trainees have been concerned with the following areas of research: (1) thyroid hormones during early development of salmonids (Greenblatt) and (2) control of prolactin and growth hormone secretion in salmon (Kelley).

Cooperating Organizations
Humboldt State University
Iron Gate Hatchery
Mad River Hatchery
National Marine Fisheries Laboratory, Cook, Washington
National Marine Fisheries Service, Seattle, Washington
Nimbus Hatchery
California Department of Fish and Game

Participants
Howard A. Bern, Professor of Zoology
Charles S. Nicoll, Professor of Physiology
Richard S. Nishioka, Specialist
Graham Young, Assistant Research Zoologist
Christopher L. Brown, Postgraduate Research Zoologist
B. Thrandur Björnsson, Swedish Science Research Council Postdoctoral Fellow
Stephen McCormick, NIH Postdoctoral Fellow
Richard Lin, Staff Research Associate
Michael Greenblatt, Sea Grant Trainee
Kevin Kelley, Sea Grant Trainee
Kevin Mark, Student Assistant (California Department of Fish and Game)
Wayne Leong, Student Assistant (California Department of Fish and Game)
Ken Takemura, Student Assistant (California Department of Fish and Game)
Elizabeth McGahan, Seasonal Aid (California Department of Fish and Game)
Anne McBride, Seasonal Aid (California Department of Fish and Game)

Other Scientific Collaborators
Dr. T. Cross, Farran Laboratory, Salmon Research Trust of Ireland, Newport, and Department of Zoology, University of Cork
Dr. E. G. Grau, Department of Zoology and Hawaii Institute of Marine Biology, University of Hawaii, Honolulu
Professor H. Kawauchi, School of Fisheries Sciences, Kitasato University
Professor T. Hirano, Ocean Research Institute, University of Tokyo
Dr. J. Bolton, Laboratory of Fish Physiology, INRA, Rennes
Dr. M. Iwata, Otsuchi Marine Research Center, University of Tokyo
Dr. D. S. King, Department of Genetics, University of California, Berkeley
Dr. P. Prunet, Laboratory of Fish Physiology, INRA, Rennes
P. Siri, Laboratory Manager, Bodega Marine Laboratory, University of California
Professor T. Kerstetter, Department of Biology, Humboldt State University, Arcata
Dr. J. Specker, Department of Zoology, University of Rhode Island
Dr. K. Yamauchi, Department of Biology, Hokkaido University, College of Fisheries, Hakodate
Dr. W. Dickhoff, School of Fisheries, University of Washington
Dr. Craig Sullivan, School of Fisheries, University of Washington
Dr. E. Plisetskaya, Department of Zoology, University of Washington
Dr. W. Zaugg, National Marine Fisheries Services, Cook, Washington
B. Barngrover, Manager, Mad River Hatchery, California Department of Fish and Game
M. Cozart, Fish Culturist, Merced River Fish Facilities, California Department of Fish and Game
C. Hiser, Manager, Iron Gate Hatchery, California Department of Fish and Game
P. Hubbell, Senior Fisheries Biologist and Leader of Research Program, California Department of Fish and Game
R. Ducey, Manager, Nimbus Hatchery, California Department of Fish and Game
T. Curtis, Associate Fisheries Biologist, California Department of Fish and Game
W. Loudermilk, Associate Fisheries Biologist, Central Valley, California Department of Fish and Game
J. White, Fisheries Biologist, Central Valley, California Department of Fish and Game

Publications
Bern, H. A., C. S. Nicoll, R. S. Nishioka, and G. Young. 1986. Endocrine control of salmonid development and seawater adaptation. In *California Sea Grant Biennial Report, 1982–84.* Publ. No. R-CSGCP-020. California

Sea Grant College Program. University of California, La Jolla. pp. 110–113.

Björnsson, B. Th., K. Yamauchi, R. S. Nishioka, L. J. Deftos, and H. A. Bern. 1987. Effects of hypophysectomy and subsequent hormonal replacement therapy on hormonal osmoregulatory status of coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 68:421–430.

Bolton, J. P., G. Young, R. S. Nishioka, T. Hirano, and H. A. Bern. 1987. Plasma growth hormone levels in normal and stunted yearling coho salmon, *Oncorhynchus kisutch*. *J. Exp. Zool.* 242:379–382.

Brown, C. L., C. V. Sullivan, H. A. Bern, and W. W. Dickhoff. 1987. Occurrence of thyroid hormones in early developmental stages of teleost fish. *Am. Fish. Soc. Symp.* 2:144–150.

Iwata, M., R. S. Nishioka, and H. A. Bern. 1987. Whole animal transepithelial potential (TEP) of coho salmon during the parr-smolt transformation and effects of thyroxine, prolactin and hypophysectomy. *Fish Physiol. Biochem.* 3:25–38.

Iwata, M., S. Komatsu, N. L. Collie, R. S. Nishioka, and H. A. Bern. 1987. Ocular cataract and incomplete seawater adaptation in salmonids. *Aquaculture* 66:315–327.

Kobuke, L., J. L. Specker, and H. A. Bern. 1987. Thyroxine content of eggs and larvae of coho salmon, *Oncorhynchus kisutch*. *J. Exp. Zool.* 242:89–94.

Nicoll, C. S., S. S. Steiny, D. S. King, R. S. Nishioka, and G. L. Mayer, N. Eberhardt, J. Baxter, M. Yamanaka, J. Miller, J. Seilhamer, J. Schilling, and L. Johnson. 1987. The primary structure of coho salmon growth hormone and its cDNA. *Gen. Comp. Endocrinol.* 68:387–399.

Nishioka, R. S., E. G. Grau, K. V. Lai, and H. A. Bern. 1987. Effect of thyroid-stimulating hormone on the physiology and morphology of the thyroid gland in coho salmon, *Oncorhynchus kisutch*. *Fish Physiol. Biochem.* 3:63–72.

Nishioka, R. S., N. H. Richman, G. Young, P. Prunet, and H. A. Bern. 1987. Hypophysectomy of coho salmon *(Oncorhynchus kisutch)* and survival in fresh water and seawater. *Aquaculture.* 65:343–352.

Richman, N. H., and W. S. Zaugg. 1987. Effects of cortisol and growth hormone on osmoregulation in pre- and desmoltified coho salmon *(Oncorhynchus kisutch)*. *Gen. Comp. Endocrinol.* 65:189–198.

Richman, N. H., R. S. Nishioka, G. Young, and H. A. Bern. 1987. Effects of cortisol and growth hormone replacement on osmoregulation in hypophysectomized coho salmon *(Oncorhynchus kisutch)*. *Gen. Comp. Endocrinol.* 67:194–201.

Richman, N. H., S. Tai de Diaz, R. S. Nishioka, P. Prunet, and H. A. Bern. 1987. Osmoregulatory and endocrine relationships with chloride cell morphology and density during smoltification in coho salmon *(Oncorhynchus kisutch)*. *Aquaculture.* 60:265–285.

Sheridan, M. 1987. Effects of epinephrine and norepinephrine on lipid mobilization from coho salmon liver incubated *in vitro*. *Endocrinology* 120:2234–2239.

Sheridan, M. A., and H. A. Bern. 1986. Both somatostatin and the caudal neuropeptide, urotensin II, stimulate lipid mobilization from coho salmon liver incubated *in vitro*. *Regul. Pep.* 14:333–344.

Sheridan, M. A., E. M. Plisetskaya, H. A. Bern, and A. Gorbman. 1987. Effects of somatostatin-25 and urotensin II on lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 66:405–414.

Specker, J. L., and L. Kobuke. 1987. Seawater-acclimation and the thyroidal response in juvenile coho salmon *(Oncorhynchus kisutch)*. *J. Exp. Zool.* 24:327–332.

Winans, G. A., and R. S. Nishioka. 1987. A multivariate description of change in body shape of coho salmon *(Oncorhynchus kisutch)* during smoltification. *Aquaculture.* 66:235–245.

Young, G., and R. J. Lin. 1988. Response of the interrenal to adenocorticotropic hormone after short-term thyroxine treatment of coho salmon *(Oncorhynchus kisutch)*. *J. Exp. Zool.* 245:35–58.

Young, G., R. S. Nishioka, and H. A. Bern. 1988. Endocrine physiology of salmon development and seawater adaptation. In *Proceedings 1st Congress of the Asia & Oceania Soc. of Comp. Endocrinol.* Ohnishi, Nagahama, and Ishizaki, eds. Nagoya University Corporation, Nagoya, Japan. pp. 363–364.

Biochemical Indices of Activity in the Northern Anchovy, *Engraulis mordax*

University of California, San Diego
R/F-102
Project Initiated: October 1, 1984
Project Completed: September 30, 1987

George N. Somero

The goal of our project was to investigate the feasibility of using the physiological adaptation of the swimming muscles to follow whole-fish energetic costs of locomotion in the northern anchovy, *Engraulis mordax*. There was evidence at the inception of this project that such a goal was realistic, based on earlier studies with mammals (Holloszy and Booth, 1976) and fish (Childress and Somero, 1979; Johnston and Moon, 1980a, 1980b; Somero and Childress, 1980; Sullivan and Somero, 1980). This likelihood has

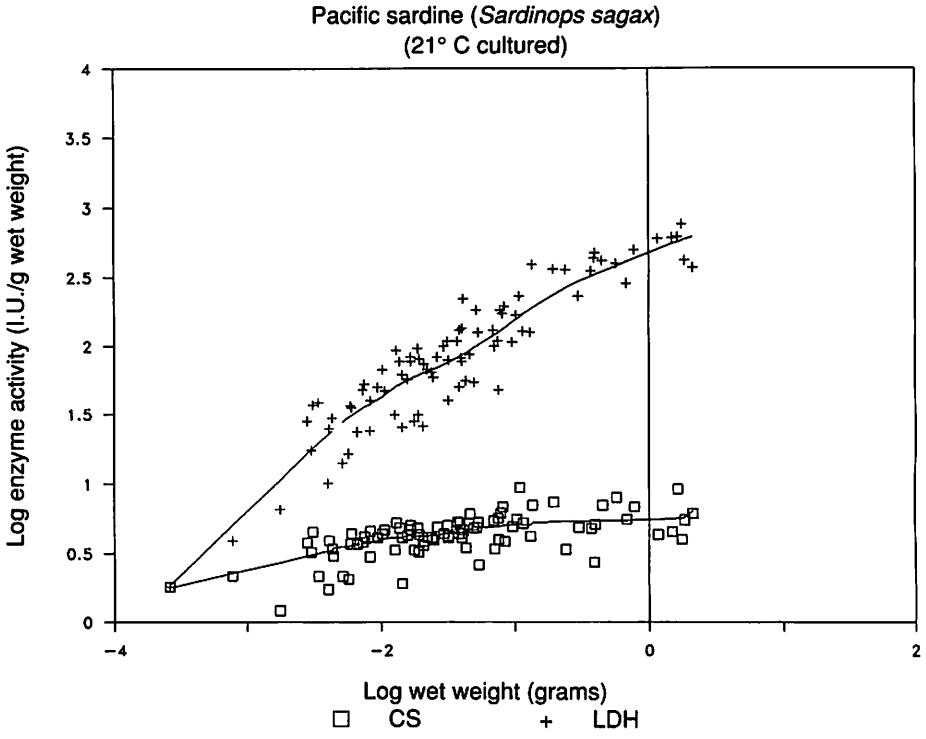


Figure 1, part 1

Figure 1. Log of enzyme activity as a function of whole-fish fresh weight for seven species of pelagic fish. The activity of lactate dehydrogenase (LDH) is greater than that of citrate synthase (CS) except near the weight of the first-feeding larvae, below 1 mg. The series smoothed lines on all graphs is that from sardine enzyme activities. Species abbreviations: S.j = *Scomber japonicus* or chub mackerel, A.n = *Atractosion nobilis* or striped bass, L.t. = *Leuresthes tenuis*, or grunion, M.c = *Mugil cephalus* or stiped mullet, and O.m. = *Oreachromis mossambica*, or tilapia.

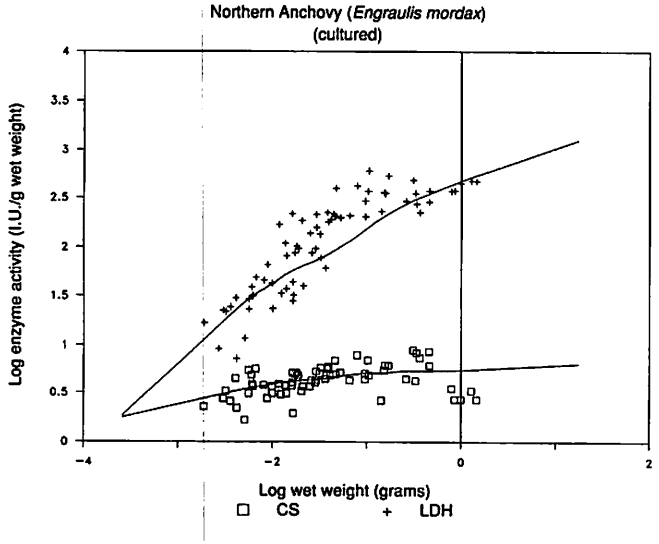


Figure 1, part 2

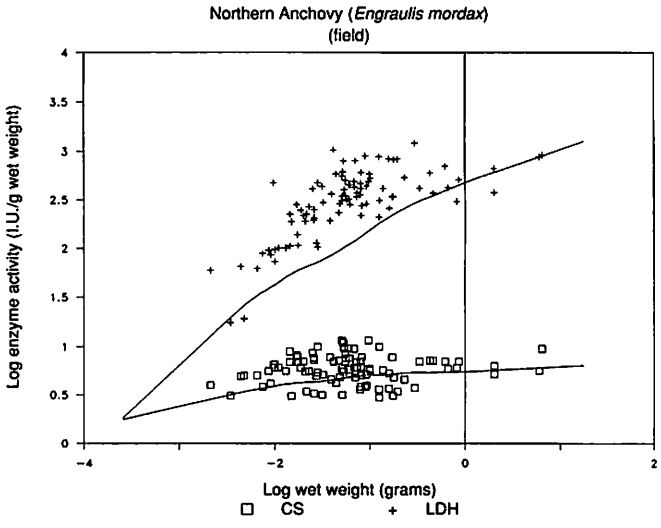


Figure 1, part 3

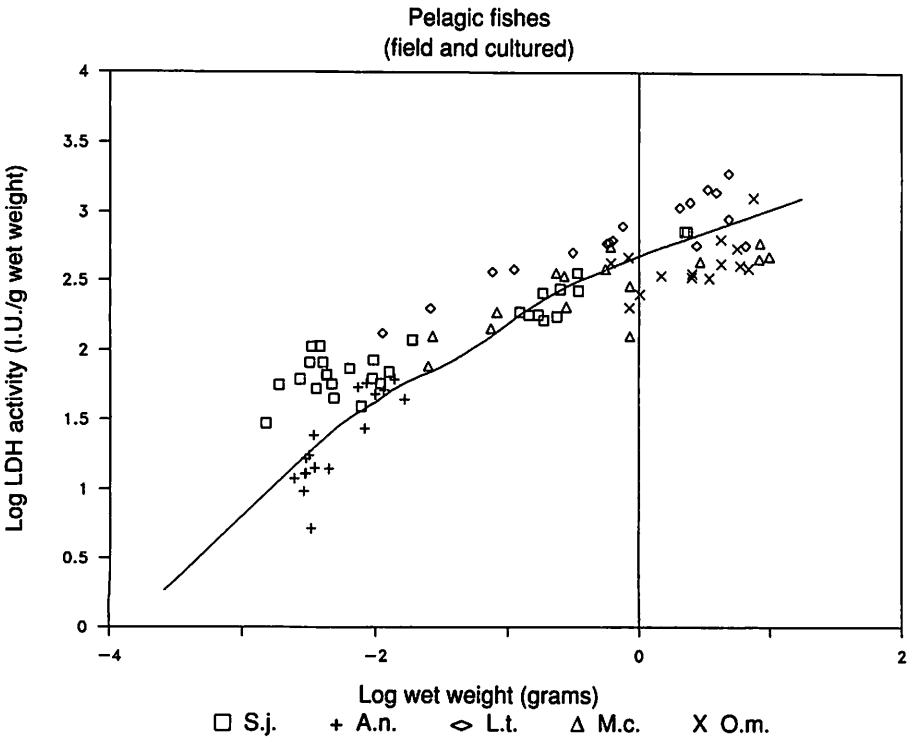


Figure 1, part 4

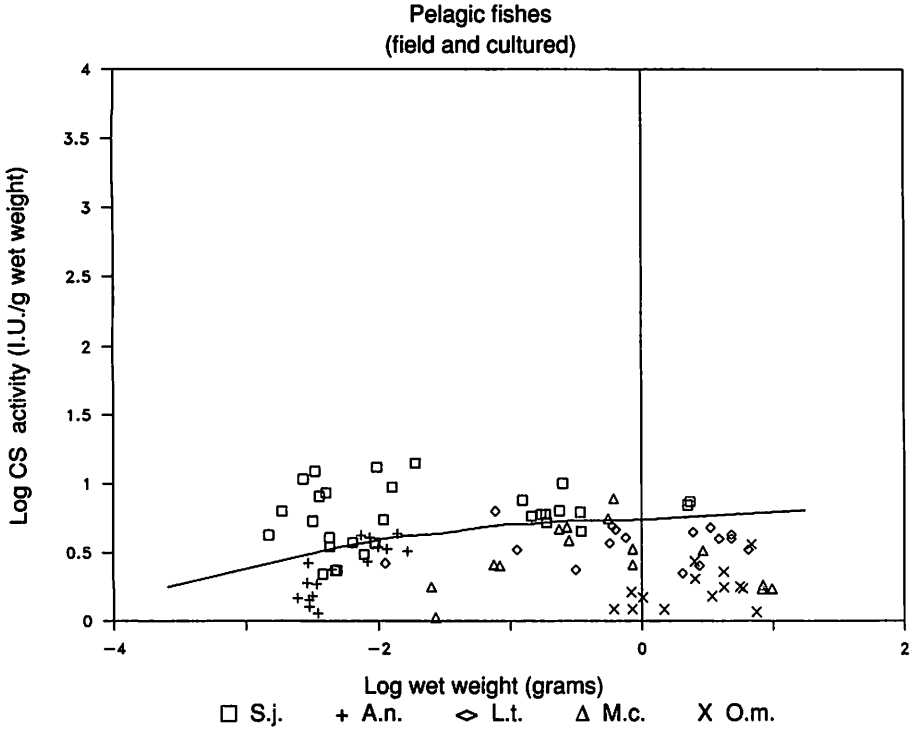


Figure 1, part 5

been bolstered further by the critical study of the correlation between metabolic enzyme activity of the trunk muscles and measured levels of whole-fish routine metabolism (Torres and Somero, 1988). We intended to follow biochemical indices of physiological change in muscle tissue of the anchovy under various exercise and growth regimens. The assessment of relevant biochemical constituents (enzymes and nucleic acids) was directed toward those types of measurements that could be performed accurately on muscle samples from fish frozen after capture in the field. Our objective, then, was to calibrate biochemical indices against locomotory activity in laboratory swimming tunnels and apply these same indices to field-collected fish.

The analysis of activity through assessment of enzyme activities in muscle could not be accomplished without the simultaneous assessment of growth rate, as we suspected that growth would influence the levels of metabolic enzymes. This influence is, in fact, an asset to our analysis. With an ability to measure growth rate accurately and our ability to measure the energetic costs of routine activity, or routine metabolism, we could construct an energy budget (metabolism + growth) for a nonreproducing fish. The quantification of growth was again achieved through the measurement of the biochemical constituents (nucleic acids) of the muscle. The concentration of the nucleic acids can be measured accurately from properly frozen tissue obtained from field surveys.

These measurements of the muscle would be applicable to any phase of development of the fish and generally applicable to any species of fish. It is this potential that has led us to investigate changes in these biochemical indices during the entire life history of the northern anchovy and parts of the life history of 11 other species of fish. We have concluded that our simple indices (metabolic enzymes

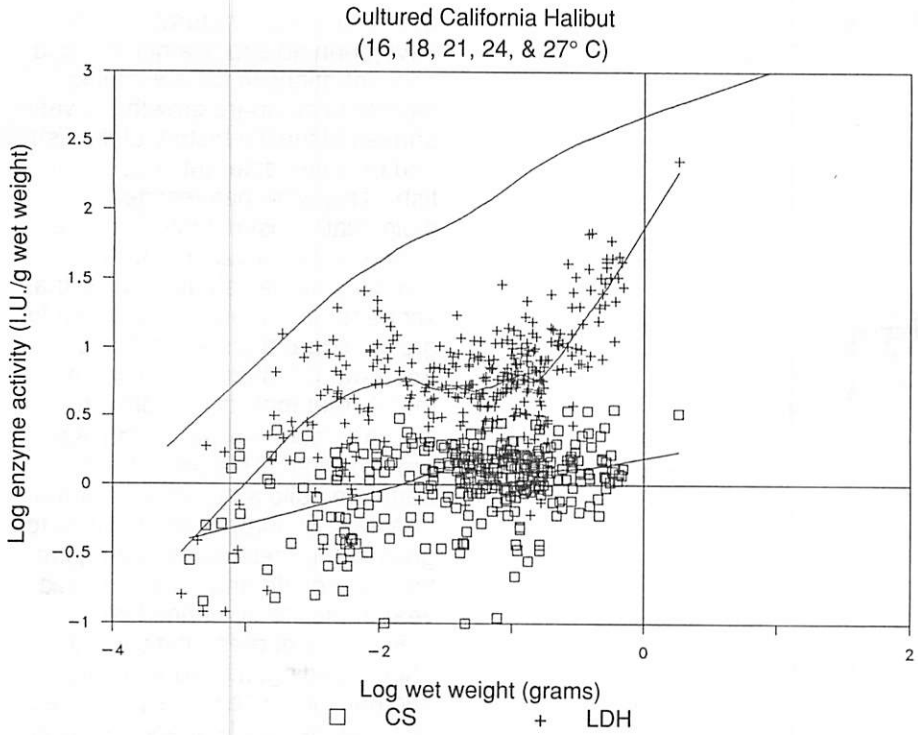


Figure 2, part 1

Figure 2. Log of enzyme activity as a function of body weight for 4 species of flatfish. Legends are the same as in Figure 1.

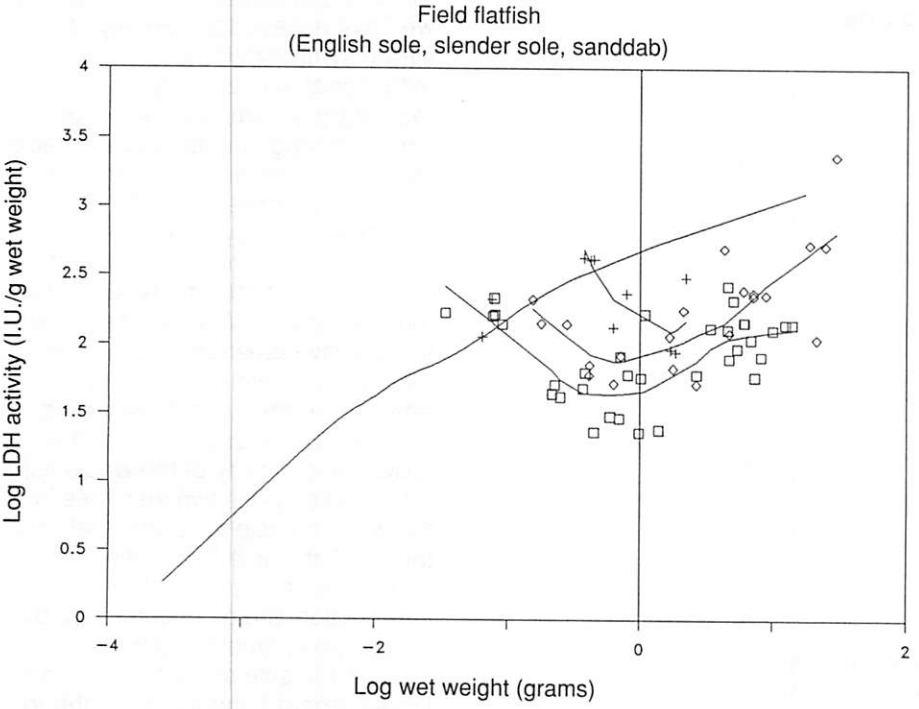


Figure 2, part 2

of the "white" trunk muscles) of whole-fish routine metabolism can be used in a size-independent manner and that our index of whole-fish growth rate (concentrations of ribonucleic acid [RNA] and deoxyribonucleic acid [DNA] in the same muscle tissue) can be normalized on a whole-fish fresh-weight basis. We think that by using these different measurements, it will be possible to assess a complete energy budget for a nonreproducing field fish.

Project Outline

The bulk of the effort in this project has been spent in the performance of a "treadmill" experiment with adult northern anchovy. The anchovys swim at a set speed (determined by the flow speed of the water) in a water channel and are fed a certain ration. This process induces a certain amount of conditioning of the swimming muscle and results in a prescribed level of growth (positive or negative). The level of routine metabolism and growth is estimated directly and used to calibrate the indices of metabolism and growth we are developing. This view of the day-to-day changes in these measurements during the life history of the fish is developing slowly because of the time required for analysis of many factors of each fish and statistical analysis of the resultant data. Despite this, our preliminary results (some of which have been made with the subsamples of the eventual sample and hence are inherently less precise) have shown that changes in growth and metabolism can be tracked on a timely basis (days). Analysis of adult field anchovys has revealed large variation in biochemical indices of metabolism and growth, leading us to conclude that there are temporal changes in the energetics of the fish that we can interpret by using our indices.

In addition to the changes seen during the adult phase of life, metabolism and growth are changing during early ontogeny (i.e., development from first-feeding

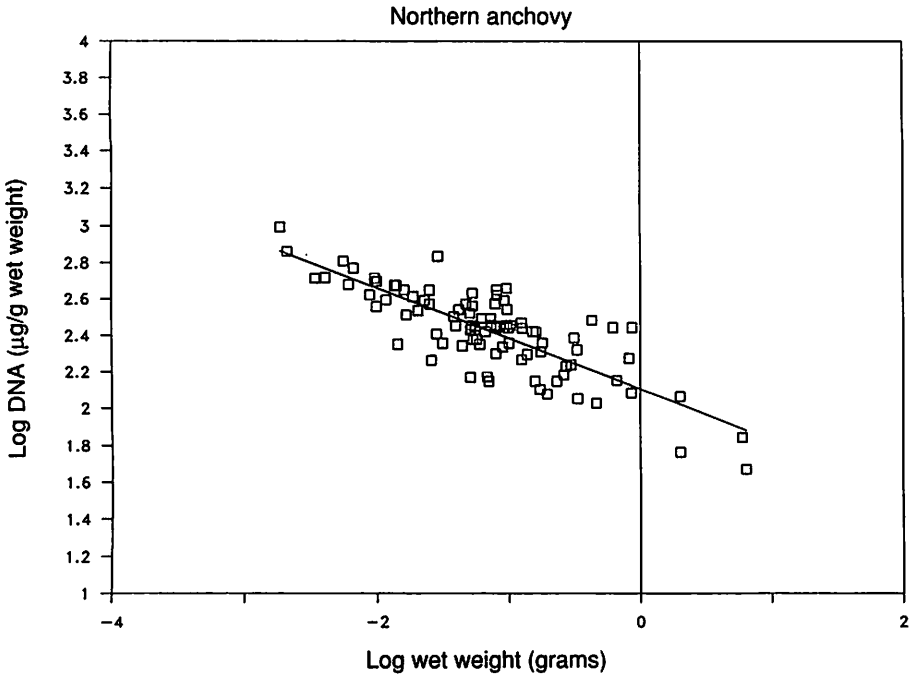


Figure 3, part 1

Figure 3. Log of the concentration of deoxyribonucleic acid (DNA) as a function of the whole-fish fresh weight. See figure 1 for pelagic species designations. The regression lines determined for anchovy is displayed on all graphs.

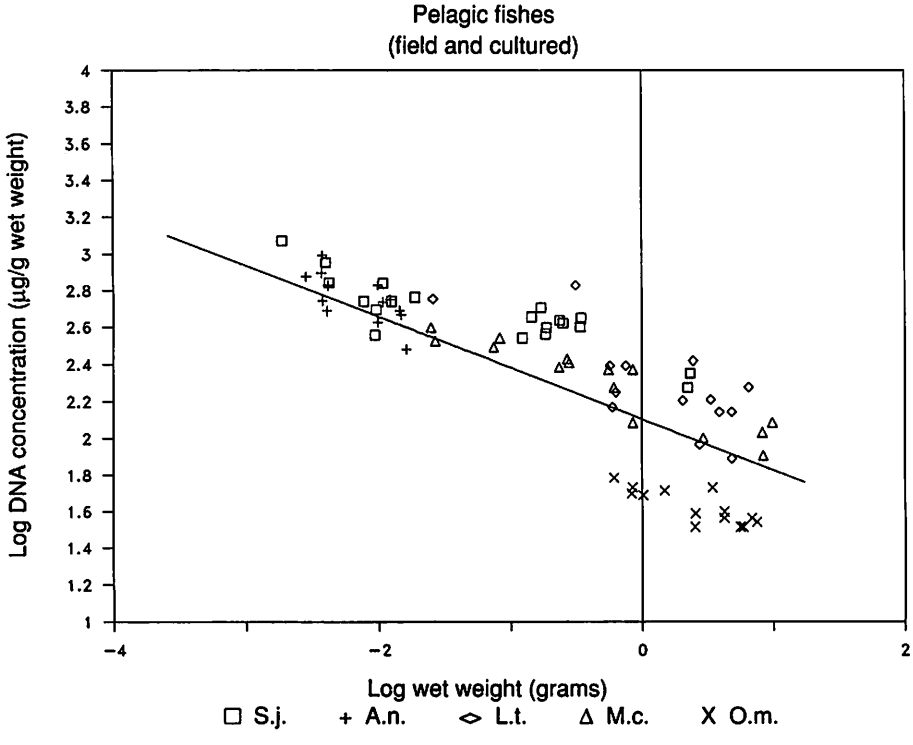


Figure 3, part 2

larvae to sexual maturation). We have been able to use nucleic acid concentrations in the swimming muscle to compare growth between phases of the life history of the fish and between different species of fish. This work has resulted in a biologically based analysis of the energetics of larval and early juvenile marine fish. It appears that scope for metabolism and scope for growth expand (as a function of fresh weight) during development, that is, metabolism and growth become more flexible as the fish grows larger. This project has begun to build a foundation for the quantitative analysis of the limits to growth and metabolism during the first year of life, the most dynamic year in the life of marine fish.

For ease of presentation and understanding, the work on larval and early juvenile fish is presented first and then preliminary results on the exercising of adult anchovy are discussed.

Changes in Biochemical Indices of Metabolism and Growth Rate

The activity of key metabolic enzymes reflects the potential and routine flow of substrate along that metabolic pathway. For this reason we have assayed the activity of citrate synthase (CS), a mitochondrial enzyme and the first regulatory enzyme in the flow of carbon through the tricarboxylic acid cycle and electron transport, and lactate dehydrogenase (LDH), a "cul-de-sac" of glycolysis that supports anaerobic production of energy on a short-term basis in the muscle tissue. We have assessed the activity (substrate that can be used per unit of time) of these enzymes in the "white" swimming muscle. This muscle reflects the growth and activity of the whole fish. The level of these two enzymes in the white muscle accurately reflects the level of whole-fish routine respiration, as noted in the introduction and as documented by Torres and Somero (1988).

From the size of the first-feeding larvae (about 1 mg fresh weight) to the end of the larval phase of life,

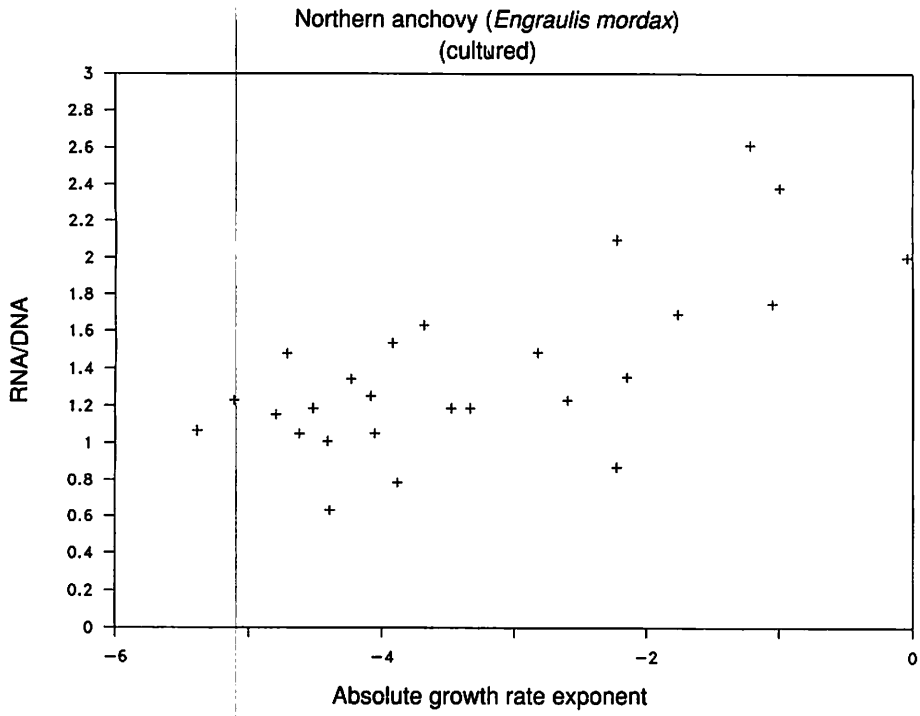


Figure 4, part 1

Figure 4. RNA/DNA ratio as a function of the exponent (using the natural log base e) describing the absolute growth rate (g/day) of the larval and juvenile fish in culture.

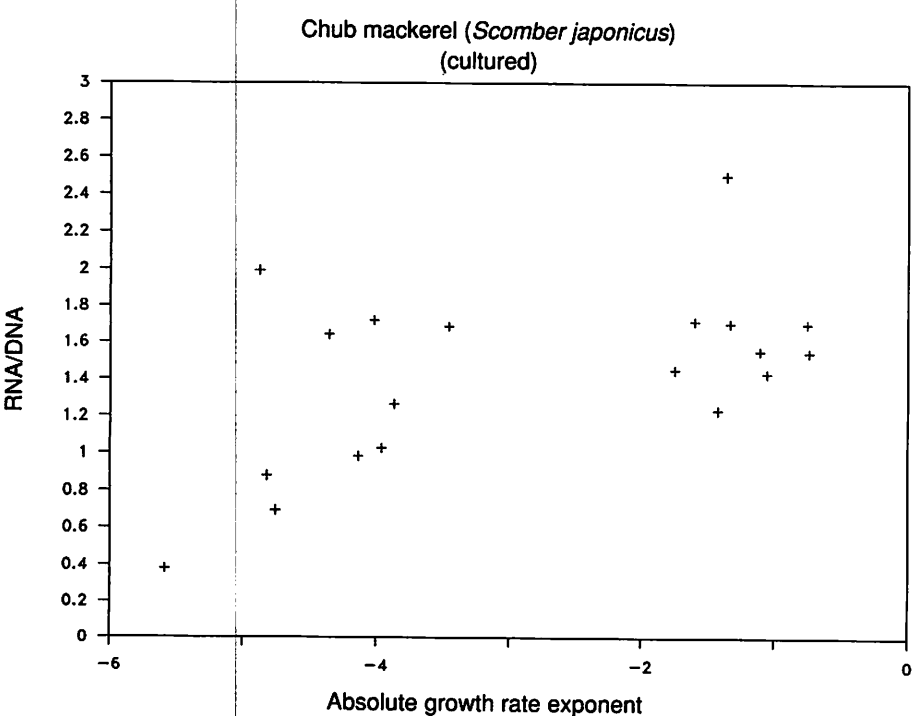


Figure 4, part 2

the routine level of respiration (weight-specific) of the northern anchovy increases steadily (see the data and regression line for CS activity [weight-specific] in Figure 1). The maximal potential energy production for swimming, hence maximal whole-fish metabolism, as represented by the activity of LDH (weight-specific), increases much faster than routine respiration, as represented by the activity of CS. The difference between routine and maximal respiration rates is a measure of scope of metabolism. Our enzymatic indices of metabolism indicate that scope increases dramatically during the larval phase of development for the northern anchovy. The scope for metabolism becomes even larger in the juvenile and adult phases, but the increase in scope with increase in weight of the fish does not appear to be as dramatic.

This same rising of routine metabolism and rapidly increasing scope for metabolism is apparent during the larval phase of six other species of fish for which we have measured CS and LDH activities of the white muscle. Five other species have been measured in the early juvenile stage, and the activites of these two metabolic enzymes are at a level similar to that found in larval fish of a similar size (see Figure 2). In the four species of flatfish analyzed during the juvenile phase, all of the fish reduce their routine metabolism and scope for metabolism in the early juvenile phase. This striking conclusion is documented in the winter flounder, where whole-fish respiration decreased (hence weight-specific respiration decreased dramatically) after transformation to juvenile stage and the adoption of a benthic existence (Laurence, 1975).

All evidence to date, obtained by us and from the scientific literature, suggests that the use of white trunk muscle metabolic enzyme activities as indices of the whole-fish rate and scope for metabolism is possible without any normalization for life stage or species-specific influence.

Buckley (1984), in reviewing his

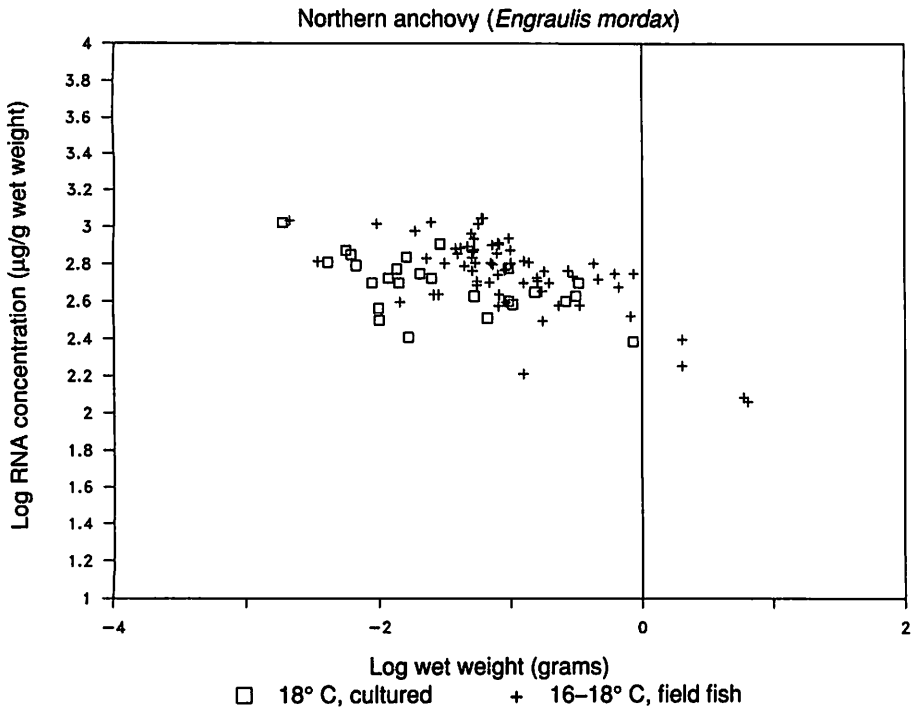


Figure 5, part 1

Figure 5. Log of the RNA concentration from the white muscle of larval and juvenile fish as a function of whole-fish fresh weight. See figure 1 for pelagic species designations.

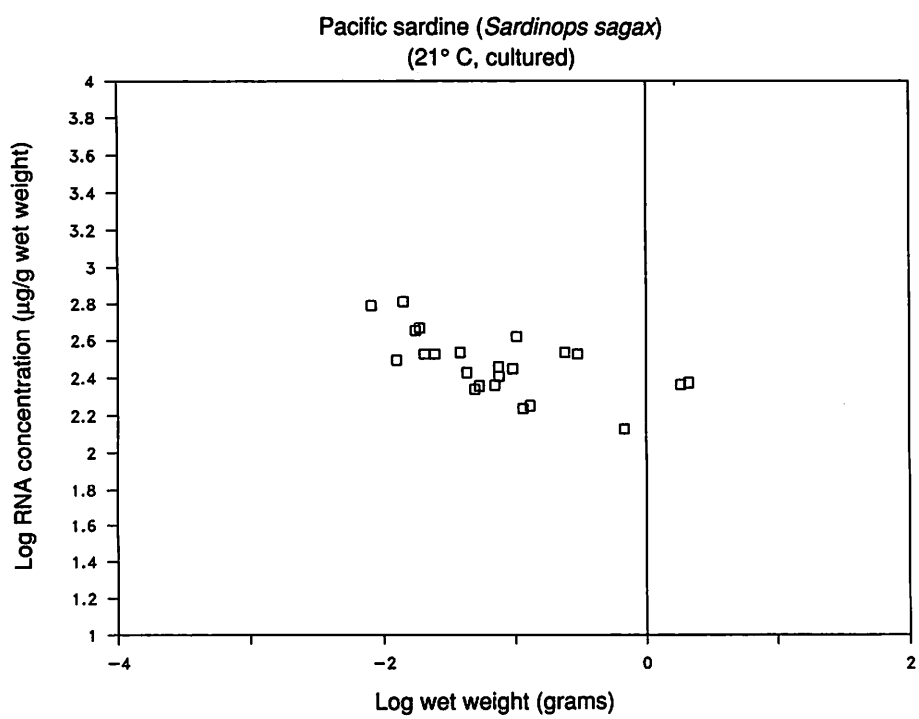


Figure 5, part 2

studies of growth on eight species of larval fish, showed good correlation between the predicted growth rate of the larvae obtained by using the concentrations of whole fish RNA and DNA and the rate actually measured. We have extended this analysis in an attempt to allow for the use of white muscle RNA/DNA ratio without calibration to laboratory experimentation. The white swimming musculature accurately reflects whole-fish growth rate and allows comparisons between phases of life and between species, once normalized for whole-fish fresh weight and species-specific DNA per nucleus. White trunk muscle fibers grow throughout the life of a fish, constantly and regularly diluting the concentration of DNA. Once adjusted for the difference in the DNA concentration per nucleus, the DNA content of the white muscle of all fish is roughly equal at a given whole-fish fresh weight. The regular decline in DNA concentration is presented for 12 species in Figure 3; this is good evidence that DNA content of the white muscle can be used to normalize growth rate as indicated by RNA concentration.

RNA/DNA ratio of the white muscles correlates positively with the absolute rate of growth in three species of cultured larval and juvenile fish (Figure 4): the northern anchovy, the chub mackerel, and the California halibut. We expect the completed analysis of our work with adult northern anchovy to extend the range of growth rate over which this analysis is calibrated.

Even though the concentration of RNA in the white muscle decreases with the size of the fish (Figure 5), the ratio of RNA to DNA increases. This correlates nicely with the increasing scope of growth (i.e., flexibilty of rate) as fish grow larger. Evidence for this can be seen in Figure 6; the RNA/DNA ratio is largest for the largest fish and decreases with increasing duration of fasting to a similar low value (about 0.5).

Our work has allowed us to develop an analysis of white-muscle nucleic acid ratio that correlates with

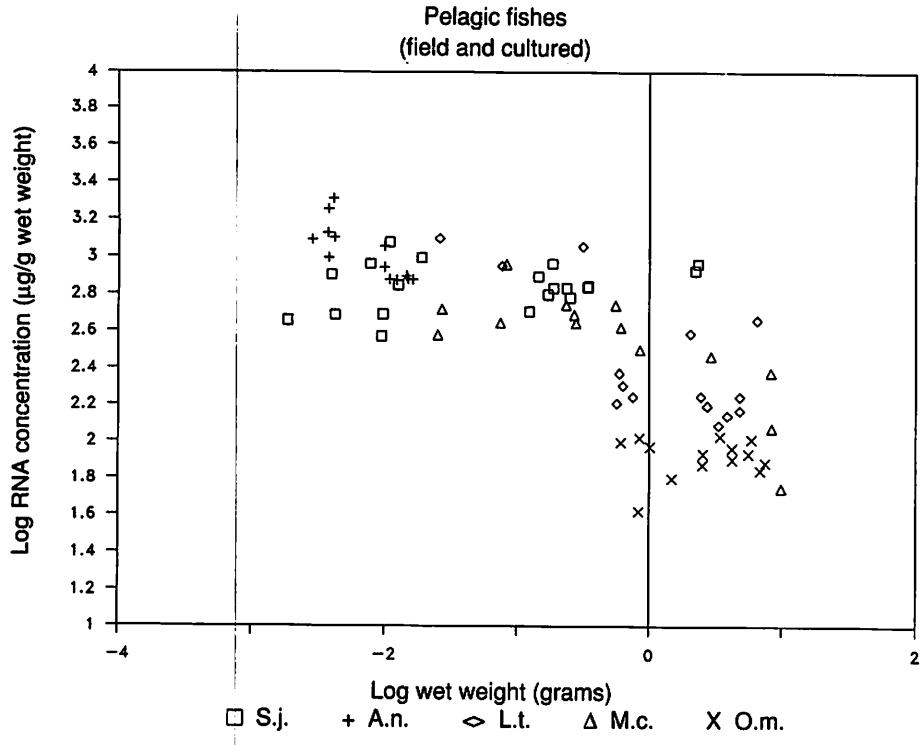


Figure 5, part 3

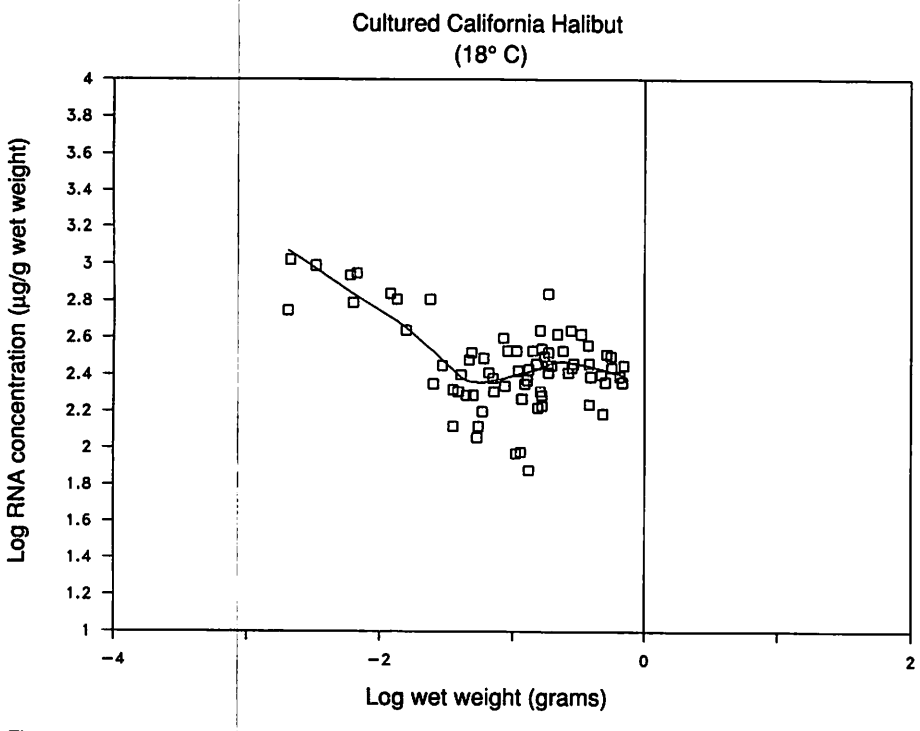


Figure 5, part 4

growth rate of the whole fish and permits comparisons of field growth on an intra- and inter-specific basis.

Effects of Exercise and Size of Ration on Biochemical Indices of Metabolism and Growth in Adult Northern Anchovy

Analysis of the activity of metabolic enzymes and nucleic acid concentration of the swimming muscles of field-caught northern anchovys has yielded a range of values of these indices (e.g., LDH activities of the white muscle that range from near 100 to about 1400 IU/g wet weight, and RNA/DNA ratios of less than 1.0 to greater than 7.0). Our experimental manipulations of adult fish have yielded means (n = 10 or more) for a group response to treatment of near 0.6 to 5.5 for an RNA/DNA ratio and LDH activities of just more than 100 to nearly 600 IU/g wet weight. Preliminary findings of the current series of treatments indicate that higher values of these biochemical indices are obtainable only with very high rations. From this brief reporting of data, it can be seen that field values exceed the range of values we have been able to produce through the manipulation of ration and activity in the laboratory.

As discussed in the previous section, the growth rate and metabolism of the adult fish can be highly variable. Indices of both metabolism and growth would be expected to vary together to a greater extent than they would in small (and younger) fish. This has been shown in our data from experiments with adult anchovy. Figure 7, which is based on data from the analysis of white muscle, shows the covariance of RNA/DNA and LDH with various exercise and ration treatments. The data presented in this graph represent means of similar treatments over several series of experiments (n = 30–50 fish) and preliminary estimates of growth rates. As a consequence, the data presented cannot be considered final expression of results and are most likely not as precise as they will be as many more fish remain to be

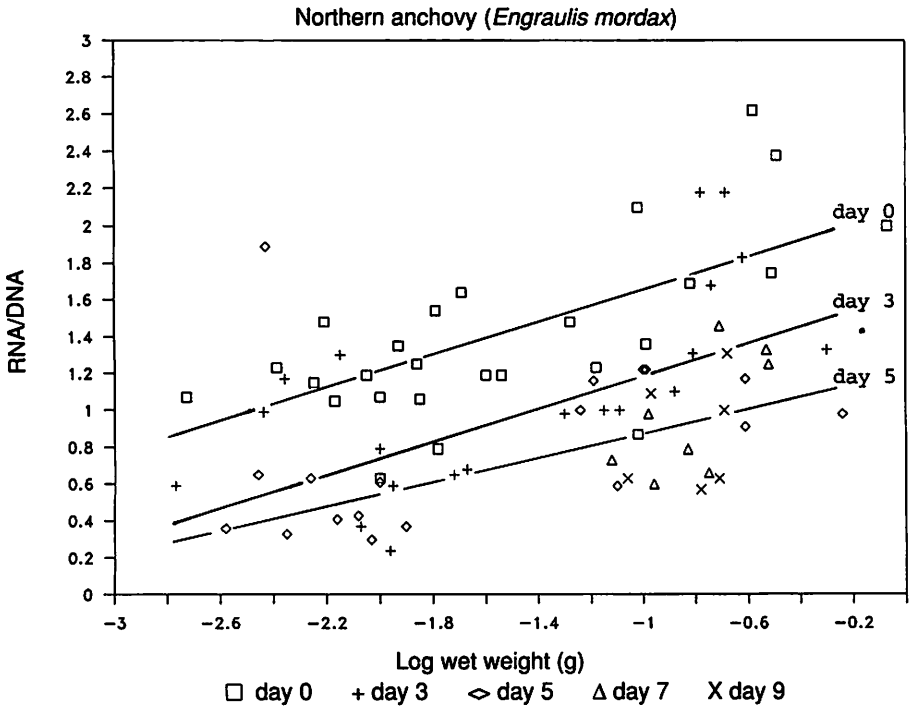


Figure 6, part 1

Figure 6. RNA/DNA ratio as a function of whole-fish wet weight and days of starvation.

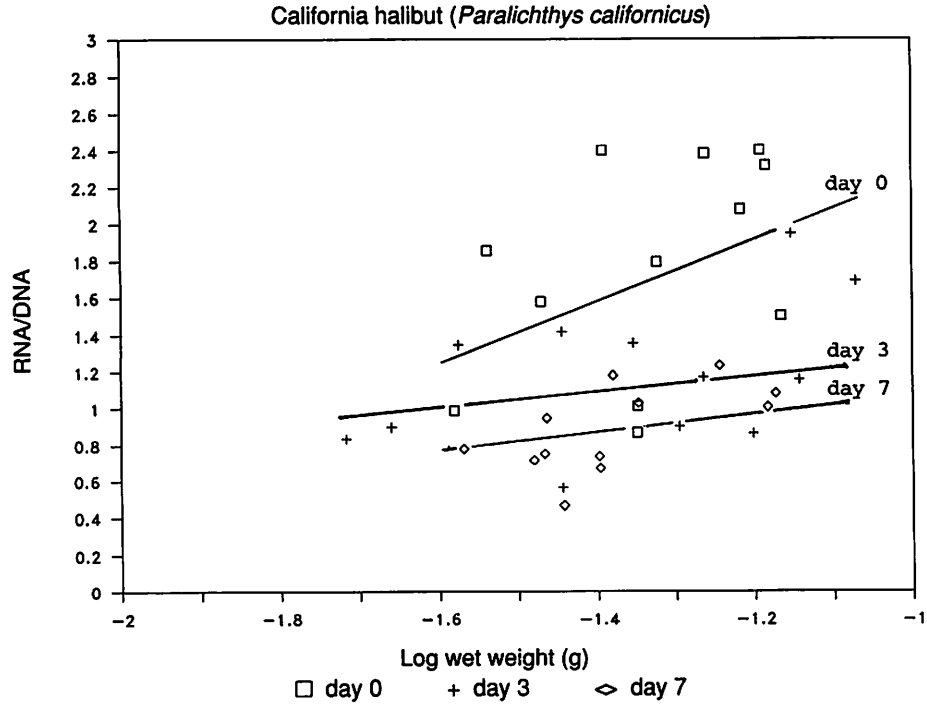


Figure 6, part 2

analyzed. Despite this, there appears to be the expected positive correlation between growth rate and RNA/DNA and some correlation between LDH activity and intensity of exercise for prolonged (3 weeks) negative growth. When a complete analysis is finished, we expect to be able to discuss accurately the precision of our combination of indices for revealing the level of metabolism and growth occurring in adult fish.

Cooperating Organizations

California Department of Fish and Game, Long Beach
Hubbs-Sea World Research Center, San Diego
Los Angeles County Museum of Natural History
National Marine Fisheries Service, Southwest Fisheries Center, La Jolla

References

Buckley, L. J. 1984. RNA-DNA ratio: An index of larval fish growth in the sea. *Mar. Biol.* 80:291–298.

Childress, J. J., and G. N. Somero. 1979. Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. *Mar. Biol.* 52:273–283.

Holloszy, J. O. and F. W. Booth. 1976. Biochemical adaptation to endurance exercise in muscle. *Ann. Rev. Physiol.* 38:273–291.

Johnston, I. A., and T. W. Moon. 1980a. Endurance exercise training in the fast and slow muscles of a teleost fish (*Pollachius virens*). *J. Comp. Physiol.* 135B:147–156.

Johnston, I. A., and T. W. Moon. 1980b. Exercise training in skeletal muscle of brook trout (*Salvelinus fontinalis*). *J. Exp. Biol.* 87:177–194.

Laurence, G. C. 1975. Laboratory growth and metabolism of the winter flounder *Pseudopleuronectes americanus* from hatching through metamorphosis at three temperatures. *Mar. Biol.* 32:223–229.

Somero, G. N., and J. J. Childress. 1980. A violation of the metabolism-size scaling paradigm: Activities of glycolytic enzymes in muscle increase in larger fish. *Physiol. Zool.* 53:322–337.

Sullivan, K. M., and G. N. Somero. 1980. Enzyme activites of fish skeletal and brain as influenced by depth of occurrence and habits of feeding and locomotion. *Mar. Biol.* 60:91–99.

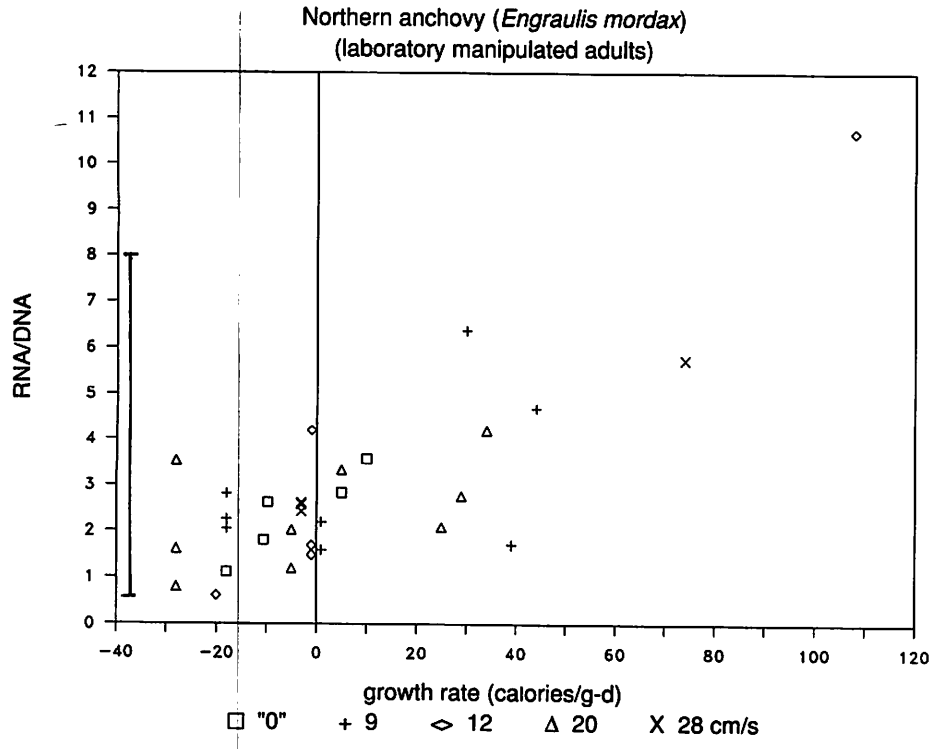


Figure 7, part 1

Figure 7. RNA/DNA ratio of the white trunk muscles of adult anchovy as a function of growth rate (top) and LDH activity as a function of metabolic rate (bottom). Left-most bar represents the range of values seen in similar sized (9-10g) field-caught fish.

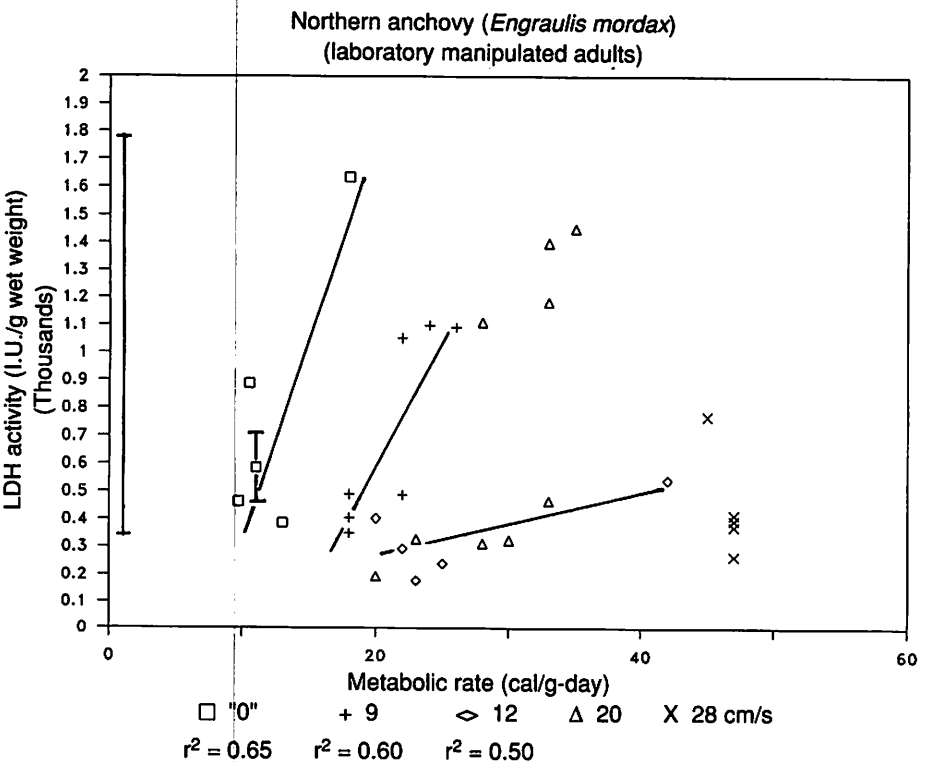


Figure 7, part 2

Torres, J. J. and G. N. Somero. 1988. Metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes. *Mar. Biol.* 98:169–180.

Publications

Kaupp, S., E. 1987. The ontogenetic development of the metabolic enzymes citrate synthase and lactate dehydrogenase in the swimming muscles of larval and juvenile fishes: A scaling of locomotory power. Master's thesis, University of California, San Diego.

Kaupp, S. E., and G. N. Somero. In prep. A scaling of metabolism during the early life history of fish. *Physiol. Zool.*

Lectures

Kaupp, S. E. What changes in the concentration of metabolic enzymes and nucleic acids can say about the life of larval and juvenile marine fish. CalCOFI conference, Lake Arrowhead, California, November 1986.

Kaupp, S. E. Fish living through chemistry: Biochemical indices of metabolism and growth in larval and juvenile marine fishes. Invited lecture, Southwest Fisheries Center, La Jolla, May 1987.

Kaupp, S. E. Limitations to growth and metabolism in larval and juvenile marine fishes. Invited lecture, Center for Scientific Investigations and Higher Education (CICESE), Ensenada, Baja California, Mexico, June 1987.

Kaupp, S. E. Biochemical indices of growth and metabolism in larval and juvenile marine fishes. Invited lecture, University of Ensenada, Ensenada, Baja California, Mexico, July 1987.

Stunting in Coho Salmon: An Investigation of Apparent Abnormalities in Ion Regulation

Humboldt State University
R/F-104
Project Initiated: October 1, 1985
Project Completed: October 31, 1987

Theodore H. Kerstetter

Introduction

The stunting phenomenon in coho salmon is a syndrome characterized by cessation of growth, retention of (or reversion to) freshwater markings, and high mortality of affected individuals (Folmar et al., 1982). It manifests itself several weeks after smolts or presmolts are transferred to seawater after initial rearing in hatcheries, and it affects a variable fraction of the population.

Research into the causes of stunting is closely related to a number of recent investigations on the role of the endocrine system in smoltification and saltwater adaptation (Clarke and Nagahama, 1977; Nishioka et al., 1982). There is no question that endocrine dysfunction is involved, but the primary reason, the initial interaction between the fish and the saltwater environment that ultimately leads to stunting, is unknown.

If juvenile coho salmon are kept in fresh water, stunting does not occur. Moreover, when stunted coho are returned to fresh water, they resume growth (Folmar et al., 1982). Clearly it is the saltwater itself that induces the syndrome in susceptible fish. Curiously, concentrations of major electroytes in the plasma are not abnormally high (Clarke and Nagahama, 1977; this study), so extracellular ionic regulation does not seem to be the cause. However, there is evidence that *intracellular* concentrations of electrolytes may be abnormally high in stunts. We earlier reported a slight but significant (10%) rise in intracellular potassium in the livers of stunts (Marini and Kerstetter, 1982). In more recent (1984) Sea Grant–supported research, we found that concentrations of both potassium and sodium were significantly higher in white muscle of stunts (Kerstetter, 1985). The research reported here extends our observations of these phenomena, describes the time course of their development in saltwater, and analyzes the effect of preadaptation in half-strength seawater on the onset of stunting.

Progress to Date

Overall Project Objective. The overall project objective was to increase our understanding of how the seawater environment brings about stunting in juvenile coho salmon. To attain this goal, we designed our experiments to learn how the major cations (sodium and potassium) varied in tissue content among different age classes of fish, at different seasons of the year, during a sequence of time intervals after the fish were transferred from fresh water to seawater. The major hypothesis of this project was that a dysfunction in intracellular ion regulation triggers the stunting phenomenon and leads to the "panhypoendocrine" condition reported by Nishioka et al. (1982).

Activities

Groups of 400 juvenile coho salmon were transferred from the Humboldt State University (HSU) hatchery to 250-gal, flow-through seawater tanks at the HSU Telonicher Marine Laboratory (TML) in January, March, May, July, and October 1986 and January 1987). Each transfer consisted of two separate subgroups, one of which had been preadapted to half-strength seawater for 10 days, the other with no exposure to saltwater. Mean body weights for each experiment are summarized in Table 1.

Samples of white muscle, liver, and plasma were taken from 6 to 10 fish from each group in each experiment (except July) at intervals of 12, 24, 48, and 72 hours and 1, 3, 5, and 7 weeks after introduction of the fish to seawater. The July experiment was designed to analyze the effects of stress on seawater adaptation, and the protocol was changed to sampling at 24 and 72 hours after transfer to saltwater. In February and April 1986 and January 1987, groups of fish of similar sizes, treated as described, were transferred to cages in the Humboldt Fish Action Council's saltwater rearing pond at King Salmon (hereafter referred to as the KSP), 2 miles south of Eureka, California, on the margin of Humboldt Bay. These fish were sampled at 24 and 72 hours and 3 weeks (2 weeks in January 1987) after their introduction to seawater.

All samples were stored frozen until laboratory analyses of five

Table 1. Mean Body Weight for Each Experiment

Experiment	Duration (wk)	Mean Body Weight ± SEM (g)	Range	N
3/86, TML	7	116.5 ± 6.3	22.6–294.2	156
5/86, TML	2	157.4 ± 9.6	29.7–403.5	112
10/86, TML	7	16.2 ± 1.3	7.5– 40.1	156
1/87, TML	7	77.8 ± 4.3	14.5–195.6	160
2/86, KSP	3	26.6 ± 2.2	13.7– 97.8	37
4/86, KSP	3	51.3 ± 5.0	23.4–129.1	56
1/87, KSP	2	35.7 ± 2.5	15.4–115.6	58

Table 2. Plasma Sodium, TML, Full-Strength Seawater

Time After Transfer	3/86	N	5/86	N	10/86	N	1/87	N
12 hr	159 ± 4.6	16	158 ± 2.2	20	177 ± 4.1	20	162 ± 2.1	20
24 hr	157 ± 3.6	19	160 ± 2.3	19	168 ± 9.7	20	171 ± 3.9	20
48 hr	151 ± 5.4	11	158 ± 2.1	12	188 ± 4.7	20	169 ± 2.2	20
72 hr	155 ± 4.0	17	159 ± 2.0	20	175 ± 5.8	19	164 ± 3.0	20
1 wk	152 ± 1.6	11	151 ± 2.1	19	169 ± 3.4	19	159 ± 1.7	20
3 wk	153 ± 1.2	17	151 ± 1.1	20	159 ± 2.6	19	155 ± 4.2	20

Note. Values for preadapted and nonadapted are pooled. Mean ± SEM μEq x ml^{-1}.

variables, (muscle sodium and potassium, liver sodium and potassium, and plasma sodium) could be performed. Tissue electrolytes were extracted by incubating muscle and liver samples in 0.1 N nitric acid at 37°C for 48 hours. Extracts were diluted volumetrically and analyzed by flame photometry for sodium and potassium. The results were expressed as microequivalents per gram dry weight. Plasma samples were diluted and then analyzed by flame photometry.

In September 1986, 10 small salmon remaining from the March 1986 experiment were identified as stunts (by small size and parr marks) and were analyzed for the five variables described. In May, 1987, 10 freshwater "smolts" were taken from the Mad River State Fish hatchery and similarly analyzed. These two groups provided us with values we could compare with data from the seawater transfer experiments. Miscellaneous analytical procedures that we occasionally used in the course of this research, and statistical procedures, are described with results from those procedures.

Results

Data Analysis. For each of the five primary tissue ion analyses, we analyzed the change in mean values with time after transfer to seawater *within* each group, and we also compared corresponding sample times *between* groups. In most cases (where tests for significance showed no difference), we pooled values from preadapted (one-half seawater) and nonadapted groups and compared the pooled means. The use of pooled data, when it occurs in this report, is noted.

Between-group comparisons were done to determine the relationships between such variables as (1) body weight and age and (2) tissue ion concentrations *at equivalent times* after transfer to seawater. Within each experiment, changes in tissue ions with increasing time in seawater were analyzed and compared to detect the onset of abnormal ion levels and the rapidity of the changes. Analysis of variance and Student-Newman-Keuls multiple comparison tests were used when results from three or more sampling times and/or experimental groups were compared.

Plasma Sodium: TML and KSP. Plasma sodium (Table 2) was generally high for 2 days to 1 week after transfer to seawater, after which it decreased to less than 160 μEq x ml^{-1} by 3 weeks. Highest mean values were in October 1986, when it peaked at 188 at 48 hours after transfer and at 3 weeks was still significantly higher (159 μEq x ml^{-1}) than the January, March, and May 1986 groups. Occasionally values of more than 220 μEq x ml^{-1} were found among most groups soon after transfer. Preadaptation had little effect on the rise in plasma sodium after transfer, except at 12 and 48 hours in the October (TML) experiment, in which the levels in preadapted fish were moderately lower, and in the July stress experiment.

Muscle Sodium: TML. Muscle sodium (Table 3) fluctuated but generally was moderately higher several weeks after transfer. In many cases it declined from initially high values, then rose again (Figure 1). Seven-week values were significantly higher than 24-hour values in the March and October

Table 3. Muscle Sodium, TML, Full-Strength Seawater

Time after Transfer	3/86	N	5/86	N	10/86	N	1/87	N
12 hr	0.078 ± 0.003	16	0.073 ± 0.003	20	0.081 ± 0.003	20	0.079 ± 0.003	20
24 hr	0.070 ± 0.003	19	0.074 ± 0.003	20	0.077 ± 0.003	20	0.070 ± 0.002	20
48 hr	0.072 ± 0.003	20	0.068 ± 0.004	11	0.097 ± 0.003	20	0.068 ± 0.002	20
72 hr	0.070 ± 0.002	20	0.078 ± 0.004	19	0.091 ± 0.003	18	0.066 ± 0.002	20
1 wk	0.074 ± 0.002	20	0.071 ± 0.003	20	0.076 ± 0.002	20	0.071 ± 0.002	20
3 wk	0.083 ± 0.002	20	0.077 ± 0.002	20	0.086 ± 0.003	20	0.068 ± 0.002	20
5 wk	0.087 ± 0.087	20	—	—	0.095 ± 0.004	20	0.071 ± 0.002	20
7 wk	0.095 ± 0.003	20	—	—	0.095 ± 0.003	18	0.079 ± 0.003	20

Note. Values for preadapted and nonadapted are pooled. Mean ± SEM μEq x mg^{-1} dry weight.

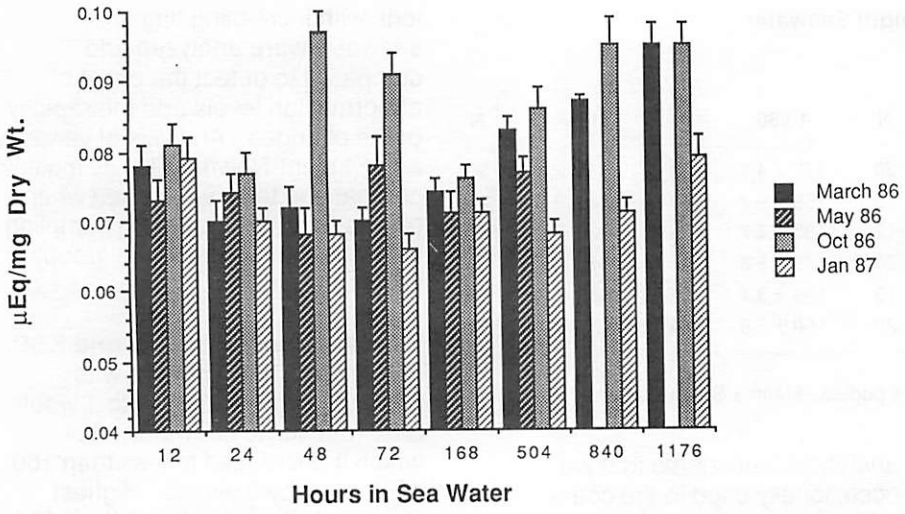


Figure 1. Muscle sodium, TML, mean ± SEM of pooled samples. Data for 840 and 1176 hours for May 1986 were not available.

1986 and January 1987 experiments. The youngest and smallest group (October 1986) showed the overall highest muscle sodium levels: 0.095 µEq x mg^{-1} by 5 weeks (compared with normal values of about 0.050 µEq x mg^{-1} from freshwater smolts).

At 7 weeks after transfer in the January 1987 experiment, a comparison of fish that weighed less than 30 g with all others gave the following results: under 30 g, muscle sodium was 0.088 µEq x mg^{-1}; more than 30 g, muscle sodium was 0.071 µEq x mg^{-1}, ($P < .001$ by Student t test). Figure 2 shows the distribution of muscle sodium and body weight for this sample.

In the October 1986 experiment only, muscle sodium correlated significantly with plasma sodium at 12, 24, 48, and 72 hours (see Figure 3) by least squares linear regressions. At 12 through 48 hours, the correlation was only for the nonadapted group, and at 72 hours only, the preadapted group correlated as above. At 1 week and succeeding sample times, levels of muscle sodium showed no apparent relationship to plasma levels but rather seemed to vary independently, dipping to a low point at 1 week (0.076 µEq x mg^{-1}) and then rising progressively to a high at 7 weeks of 0.095. The steady increase was statistically significant, indicating that a metabolic dysfunction affecting the entire group was probably the cause.

Muscle Sodium: KSP. Levels of muscle sodium from the KSP populations showed no predictable time trends. The February and April 1986 values were moderately high but steady, whereas the January 1987 values were the lowest we found, other than from freshwater controls. It is of interest that levels of muscle sodium in the January 1987 experiment showed a negative correlation with body weight (least squares, r = .46, $P < .05$), as did the January 1987 experiment at TML. Table 4 summarizes the KSP data for muscle sodium.

Muscle Potassium: TML and KSP. Levels of muscle potassium did not change in predictable patterns, although significant differences occurred between groups (Figure 4). Because fat cells have little potassium, differences in intramuscular fat can sensitively affect levels of muscle potassium. Consequently, we are unwilling to attempt an explanation for observed differences in levels of muscle potassium between groups without information on tissue fat content for the different groups.

Liver Potassium: TML. Changes in levels of liver potassium after transfer to seawater followed a consistent pattern, reaching peak levels at 48 hours (May 1986) or 72 hours (all others) and then declining (Figures 5 and 6, Table 5). Significant differences (Student-Newman-Keuls multiple comparison test) between experimental groups were evident by 72 hours (May 1986 vs. all others). At 3 weeks, differences had widened, and the

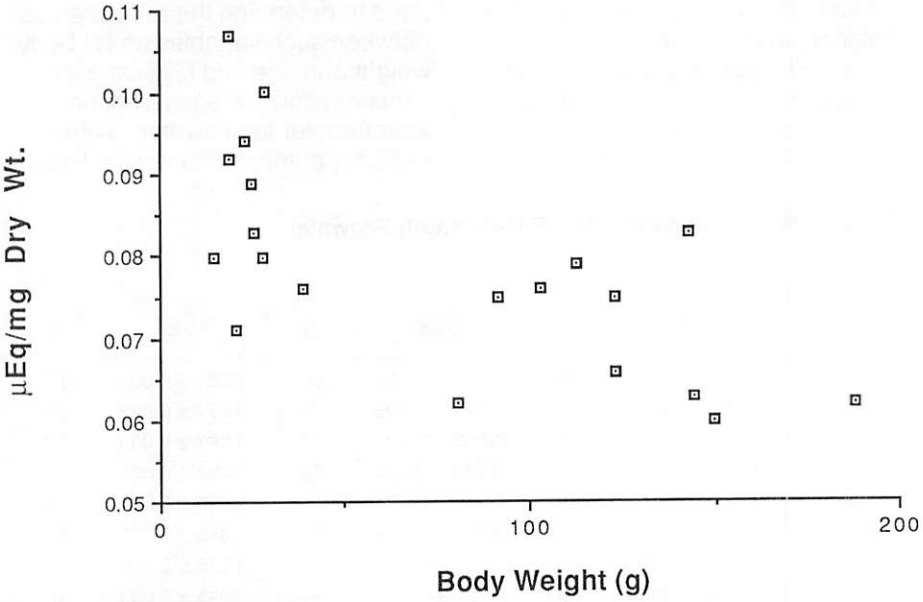


Figure 2. Muscle sodium vs. body weight January 1987, 1176 hours. Points represent individual fish.

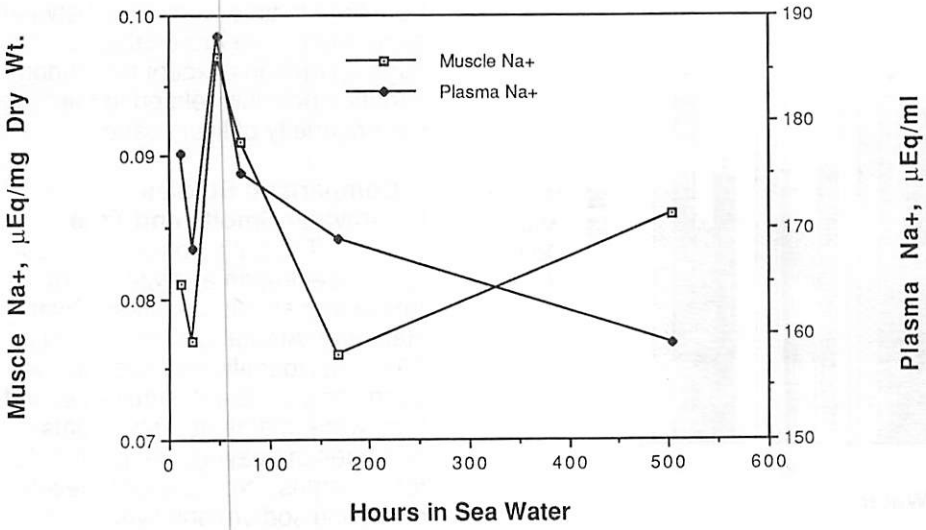


Figure 3. Muscle sodium and plasma sodium to three weeks (504 hours) for October 1987 pooled means. Correlations by least squares were significant through 72 hours (see text).

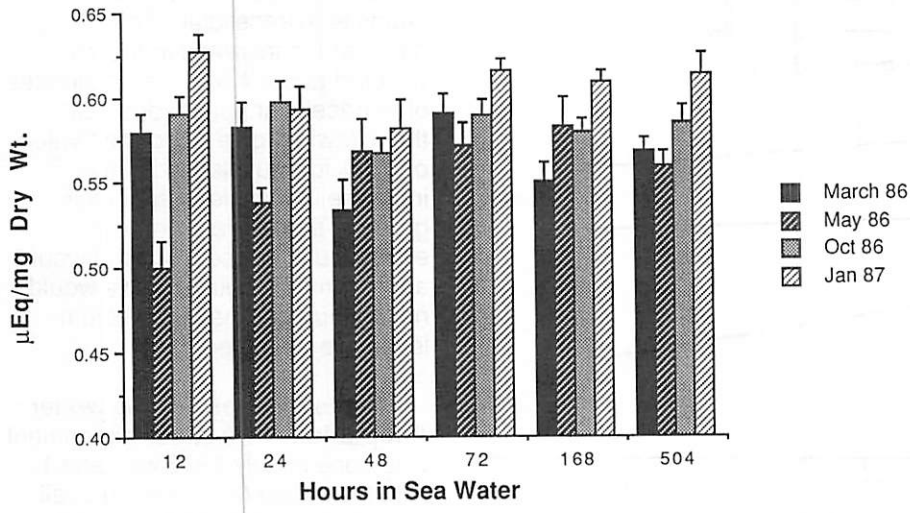


Figure 4. Muscle potassium, TML, mean ± SEM of pooled samples to 3 weeks.

four experimental groups had differentiated into three subsets: May 1986 (0.397 µEq x mg^{-1}), March 1986 plus January 1987 (0.422 and 0.434 µEq x mg^{-1}), and October 1986 (highest at 0.460 µEq x mg^{-1}).

Of interest is the difference in liver potassium values between the March 1986 and May 1986 groups at 3 weeks (Table 5), even though mean body weight was similar (Table 1). On the other hand, body weights of the March 1986 and January 1987 groups were significantly different (Student t test) although liver potassium values were not. So, size seems not to be the determining factor in potassium regulation, whereas season (month of transfer to seawater), possibly as it relates to smolt transformation, remains potentially important.

In contrast to levels of muscle sodium, levels of liver potassium did not correlate with body weight in any of our experiments. Consequently, we have concluded that whereas changes in liver potassium (in seawater) and differences between experimental groups at equivalent times after transfer to seawater are genuine and reproducible, their relationship to stunting of coho salmon is uncertain.

Liver Potassium: KSP. In the KSP experiments, liver-potassium values were generally low and did not show the rise at 72 hours typical of the TML experiments (Table 6). The two 1986 experiments yielded liver potassium values at 3 weeks of 0.36 µEq x mg^{-1} for February and April. In January 1987, liver potassium was 0.41 at 2 weeks. The latter group was comparable to the TML May 1986 experiment, the lowest in liver potassium of all the TML experiments at 3 weeks. A comparison of the January 1987 KSP and TML experiments showed that the KSP liver potassium values were significantly lower even though the mean body weights were smaller at all three sample times. This is additional evidence that size is not a primary factor in potassium regulation in saline water, but in this case the environment (outdoor and natural vs. indoor and artificial) seems to be implicated.

Liver Sodium. Variations in levels of liver sodium were not predictable and did not correlate with other variables. Although significant differences appeared

Table 4. Muscle Sodium, KSP

Time after Transfer	2/86	N	4/86	N	1/87	N
24 hr	0.092 ± 0.006	12	0.078 ± 0.003	19	0.059 ± 0.002	19
72 hr	0.083 ± 0.004	13	0.084 ± 0.004	18	0.058 ± 0.002	20
2 wk	—	—	—	—	0.052 ± 0.002	20
3 wk	0.088 ± 0.003	12	0.088 ± 0.006	15	—	—

Note. Values for preadapted and nonadapted are pooled. Mean ± SEM µEq x mg^{-1} dry weight.

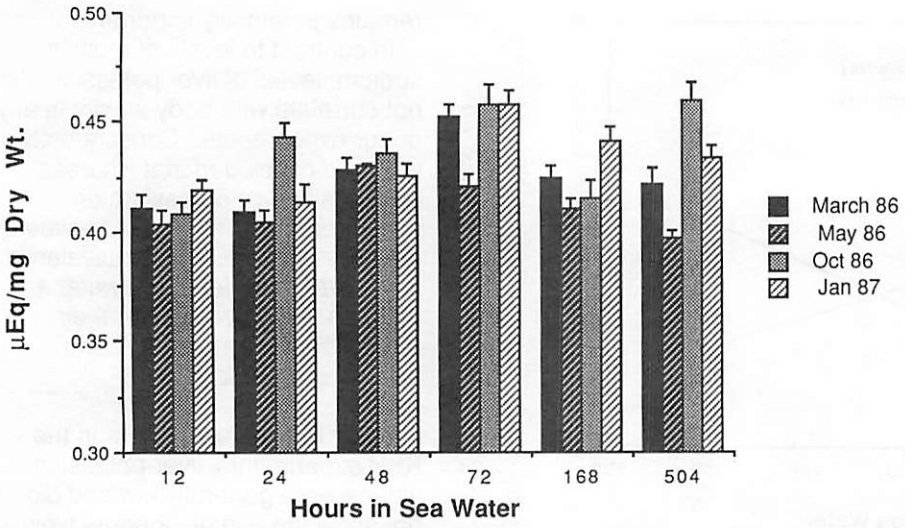


Figure 5. Liver potassium, TML, mean ± SEM of pooled samples to 3 weeks. See text for description of significant differences.

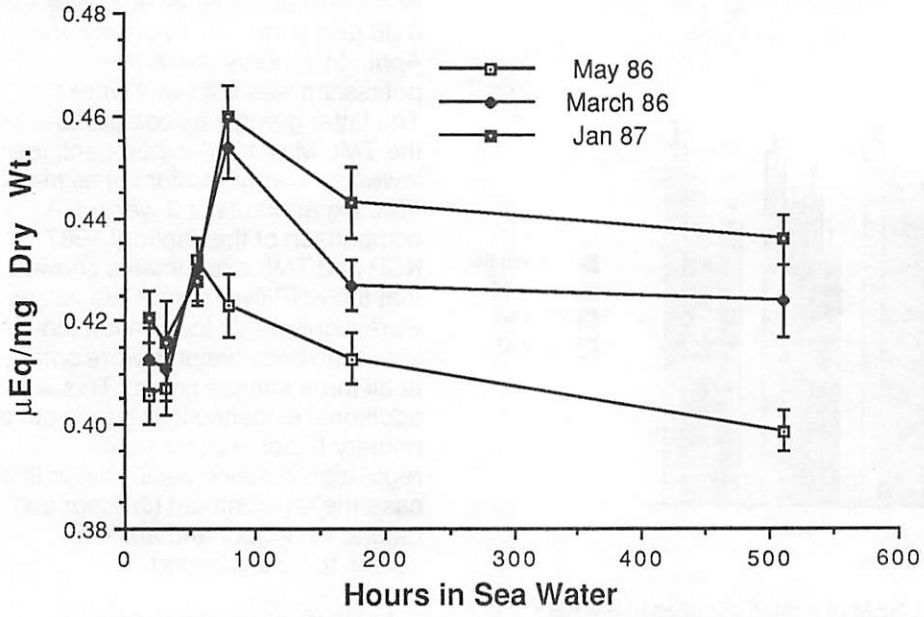


Figure 6. Time course of liver potassium in three experiments at TML, mean ± SEM of pooled samples.

Table 5. Liver Potassium, TML, Full-Strength Seawater

Time after Transfer	3/86	N	5/86	N	10/86	N	1/87	N
12 hr	0.411 ± 0.006	16	0.404 ± 0.006	20	0.408 ± 0.006	20	0.419 ± 0.005	20
24 hr	0.409 ± 0.006	19	0.405 ± 0.005	20	0.443 ± 0.007	20	0.414 ± 0.008	20
48 hr	0.428 ± 0.006	20	0.430 ± 0.011	11	0.436 ± 0.006	20	0.426 ± 0.005	20
72 hr	0.452 ± 0.006	20	0.431 ± 0.006	20	0.458 ± 0.009	20	0.458 ± 0.006	20
1 wk	0.425 ± 0.005	20	0.411 ± 0.005	20	0.416 ± 0.008	20	0.441 ± 0.007	20
3 wk	0.422 ± 0.007	20	0.397 ± 0.004	19	0.460 ± 0.008	19	0.434 ± 0.005	20
5 wk	0.387 ± 0.004	20	—	—	0.472 ± 0.009	20	0.424 ± 0.004	20
7 wk	0.413 ± 0.012	20	—	—	0.435 ± 0.010	19	0.462 ± 0.005	20

Note. Values for preadapted and nonadapted are pooled. Mean ± SEM µEq x mg^{-1} dry weight.

from time to time within and between experiments, we are unable to suggest reasons except for random variation possibly related to the heterogeneity of liver tissue.

Comparison Studies: Freshwater Smolts and True Stunts. Table 7 summarizes tissue ion concentration analyzed in 10 freshwater smolts from Mad River Hatchery, Arcata, California, and in 10 stunts, identified as such by low condition coefficient, small size, and freshwater markings. The stunts had been in seawater tanks at TML for 6 months. Note elevated levels of muscle sodium and liver potassium in the stunts and contrasting low values for these two variables in the freshwater smolts. An important question is how much of the elevated muscle sodium in stunts is extracellular. This group had a mean increase in muscle water of about 4.5%. Our estimates of extracellular space, described in the following, give a "normal" value of 4.7% for muscle, so if the increase in muscle water in this group of stunts were entirely extracellular, muscle sodium would approximately double. This would not account for the high sodium levels we observed.

Effects of Stress on Seawater Adaptation. The stress experiment was done in July 1986 with age 1+ fish, the oldest (and largest) of all fish used in this project. The fish were divided into four subgroups and treated as follows: group 1,

Table 6. Liver Potassium, KSP

Time after Transfer	2/86	N	4/86	N	1/87	N
24 hr	0.353 ± 0.008	12	0.371 ± 0.010	19	0.391 ± 0.007	20
72 hr	0.336 ± 0.007	13	0.386 ± 0.007	18	0.387 ± 0.006	19
2 wk	—	—	—	—	0.417 ± 0.006	20
3 wk	0.362 ± 0.006	11	0.358 ± 0.006	14	—	—

Note. Values from preadapted and nonadapted are pooled. Mean ± SEM μEq x mg^{-1} dry weight.

Table 7. Muscle Sodium and Liver Potassium from Seawater Stunts and Freshwater Controls

	N	Weight (g)	Condition Coefficient	Muscle Sodium	Liver Potassium
Stunts	9	31.3 ± 2.4	0.79 ± 0.03	0.224 ± 0.037	0.457 ± 0.011
Controls	10	96.0 ± 10.7	1.03 ± 0.04	0.046 ± 0.001	0.385 ± 0.006

Note. Values are mean ± SEM μEq x mg^{-1}. Condition coefficient = (weight x 100)/$length^3$.

preadapted in half-strength seawater 10 days, then chased for about 5 minutes, netted, and transferred to a tank of full-strength seawater; group 2, preadapted as group 1 but changed to full-strength seawater without capture and transfer; group 3, transferred in 30-gal containers from HSU hatchery to TML and placed directly into seawater tanks; and group 4, transferred to TML from the HSU hatchery, placed in a filtered freshwater tank at TML for 48 hours, then changed to full-strength seawater without further handling.

Stress before transfer into full-strength seawater had no apparent effect on tissue ion regulation. However, in this experiment preadaptation in half-strength seawater apparently aided some aspects of ion regulation when those fish were transferred to full-strength seawater. In the preadapted group, plasma sodium was significantly lower at 24 and 72 hours, muscle sodium was lower at 24 hours, and liver potassium was lower at 72 hours. Results are summarized in Table 8.

Table 8. Muscle and Plasma Sodium and Liver Potassium in Stressed and Nonstressed Fish after Transfer to Seawater

Hours after Transfer	Stressed	Pre-adapted	Muscle Sodium	Liver Potassium	Plasma Sodium
24	yes	yes	0.064 ± 0.006	0.423 ± 0.009[1]	179.4 ± 3.8
24	no	yes	0.063 ± 0.005	—	184.0 ± 3.5[2]
24	yes	no	0.094 ± 0.009	0.454 ± 0.008	216.8 ± 4.8
24	no	no	0.079 ± 0.009	0.443 ± 0.013	203.6 ± 9.6
72	yes	yes	0.062 ± 0.003	0.385 ± 0.006	183.0 ± 13.1
72	no	yes	0.073 ± 0.011	0.379 ± 0.010	184.5 ± 4.4
72	yes	no	0.068 ± 0.008[2]	0.435 ± 0.011[2]	213.8 ± 4.5[3]
72	no	no	0.069 ± 0.004	0.450 ± 0.008	201.4 ± 7.0[2]

Note. Mean ± SEM μEq x mg^{-1} for tissue, μEq x ml^{-1} for plasma. N = 7 except as noted. See text for procedure for stressing.

[1]N = 5.

[2]N = 6.

Estimates of extracellular space. Estimates of extracellular space were necessary to determine whether increases in tissue ion concentration resulted from changes in the percentage of tissue water that was *outside* cells. An increase in extracellular space would, for example, greatly increase the tissue sodium values because concentration of extracellular fluid is about 10 times that of intracellular fluid. Six stunts (mean body weight, 15.9 ± 1.6 g) had mean estimates of extracellular space, in ml x g^{-1} of tissue, of 0.123 ± 0.014 (SEM) and 0.058 ± 0.009 for liver and muscle, respectively. Equivalent values for six normal fish (mean body weight, 98.7 ±9.9 g) were 0.101 ± 0.008 and 0.047 ± 0.009. Differences were not statistically significant.

Conclusions

The significant findings of this project are that muscle-sodium and liver-potassium content in some juvenile coho salmon increase in the weeks immediately after their transfer to seawater. Although it is conjectural whether these findings relate to the cause(s) of stunting, it seems probable that they reflect problems in adapting to the saltwater environment and so are at least related in a general way to stunting. It is important to note that conditions of our experiments at the TML were *designed* to be suboptimal in order to induce stunting or other metabolic dysfunctions.

Levels of muscle sodium tended to increase in our laboratory seawater environment (TML). Although transient downward changes in the first few days of our experiments were sometimes evident (Figure 1), by 5 to 7 weeks the rise was apparent. In contrast, levels of muscle sodium in the two 1986 KSP experiments were moderate and steady, at least to three weeks, and moderate and

steady in the 1987 KSP experiment. Possibly, those results reflect the influence of a somewhat reduced salinity (25 ppt) in 1987. Muscle sodium values were consistently low in the freshwater controls (Table 7).

The negative correlation between low body weight and high muscle sodium in two experiments, noted earlier, and the very high values typical of extreme stunts (Table 7) are evidence that high levels of muscle sodium are characteristic of stunts. However, in the March 1986 experiment, high levels of muscle sodium did not correlate with low body weight, and in the October 1986 experiment, they correlated positively with plasma levels of sodium for 3 days (correlation coefficients of .77, .74, .72, and .53 for 12, 24, 48, and 72 hours; $P <$ .05), after which levels of muscle sodium rose while levels of plasma sodium fell. So, the causes of high muscle sodium seem varied, and attributing the condition *only* to stunting is not warranted.

In the more natural salinity environment of the KSP experiments, levels of liver potassium were significantly lower (as was the case with the January 1987 level of muscle sodium). Thus, environmental conditions seem to bring on (or contribute to) high levels of liver potassium. Two possible physiological explanations are an increased activity of sodium–potassium activated adenosine triphosphatase in hepatocyte membranes and a dysfunction in seawater absorption from the gut, leading to abnormally high salt loads in the hepatic portal system. Either occurrence could cause potassium loading of liver cells. Collie and Bern (1982) and Loretz et al. (1982) have described changes in intestinal water absorption that normally occur in coho salmon in preparation for seawater entry. If these changes are not complete when the fish enter seawater, it is reasonable to expect osmotic and ionic regulatory problems. Whatever the cause, the appearance of high levels of liver potassium in the hours or days after transfer of juvenile salmon to seawater may be the earliest available indicator that ionic regulation in saltwater is not proceeding normally.

Cooperating Organizations

California Department of Fish and Game, Mad River Hatchery, Arcata, California.

Humboldt Fish Action Council, Eureka, California.

References

Clarke, W. C. and Y. Nagahama. 1977. Effect of premature transfer on growth and morphology of the pituitary, thyroid, pancreas, and interrenal in juvenile coho salmon (*Oncorhynchus kisutch*). *Can. J. Zool.* 55:1620–1630.

Collie, N. L., and H. A. Bern. 1982. Changes in intestinal fluid transport on growth and morphology of the pituitary, thyroid, pancreas, and interrenal in juvenile coho salmon (*Oncorhynchus kisutch*). *J. Fish Biol.* 21:337–348.

Folmer, L. C., W. W. Dickhoff, C. V. W. Mahnken, and F. W. Waknitz. 1982. Stunting and parr-reversion during smoltification of coho salmon (*Oncorhynchus kisutch*). Aquaculture 28:91–104.

Kerstetter, T. H. 1985. An investigation of the physiological cause of parr-reversion in coho salmon (*Oncorhynchus kisutch*). In *California Sea Grant College Program, 1982–84 Biennial Report.* California Sea Grant College Program, La Jolla.

Loretz, C. A., N. L. Collie, N. H. Richman, and H. A. Bern. 1982. Osmoregulatory changes accompanying smoltification in coho salmon. Aquaculture 28:67–74.

Marini, G. and T. H. Kerstetter. 1982. Intracellular electrolyte regulation in freshwater and seawater-adapted coho salmon. *Aquaculture* 28:75–80.

Nishioka, R. S., H. A. Bern, K. V. Lai., Y. Nagahama, and E. B. Grau. 1982. Changes in the endocrine organs of coho salmon during normal and ahnormal smoltification—an electron microscope study. *Aquaculture* 28:21–38.

Nishioka, R. S., G. Young, E. B. Grau, H. A. Bern. Environmental and endocrine bases for lunar-phase hatchery release of salmon. Presented at the 2nd North Pacific Aquaculture Symposium, Tokai University, Tokyo, Japan, 1983.

Conferences

Kerstetter, T. H. Intracellular electrolyte abnormalitites in stunted coho salmon. Presented at West Coast Smolt Transformation Workshop, National Marine Fisheries Service, Northwest and Alaska Fisheries Center, Seattle, Washington, October 1986.

Kerstetter, T. H. Stunting in coho salmon: Elevated liver potassium following transfer of juveniles to seawater. Presented at West Coast Smoltification Workshop, University of California, Berkeley, October 1987.

Ceratomyxa shasta: Geographic and Seasonal Distribution, Salmon Strain Susceptibility and Transmission

Humboldt State University
R/F-105
Project Initiated: October 1, 1985
Project Completed: December 31, 1987

Gary L. Hendrickson

Ceratomyxosis is a disease of trout and salmon in the Pacific Northwest caused by the myxozoan *Ceratomyxa shasta*. Ceratomyxosis has caused disease and deaths in hatchery and in wild salmonids, both juvenile and adult (Rucker et al., 1954; Wales and Wolf, 1955; Conrad and DeCew, 1966; Schafer, 1968; Sanders et al., 1970; Sanders et al., 1972; Ratliff, 1981; Buchanan et al., 1983). Salmonid species and strains vary widely in susceptibility to ceratomyxosis (Schafer, 1968; Johnson, 1975; Zinn et al., 1977; Ratliff, 1981; Buchanan et al., 1983; Ching, 1984; Ching and Munday, 1984b). Within a species or strain, susceptibility may vary from highly susceptible to resistant.

The life cycle of *C. shasta* is not completely known, but its ecology and infection process have been studied. The geographic distribution of *C. shasta* is limited to northern California, Oregon, Washington, Idaho, and British Columbia (Johnson et al., 1979; Ching and Munday, 1984a). Within its geographic range, only certain waters contain the infective stage. Other waters may contain infected salmonids but lack the infective stage (Johnson, 1975; Sanders et al., 1970; Johnson et al., 1979). Little work has been done on the geographic distribution of the infective stage of *C. shasta* in California (Table 1). In particular, nothing has been reported about rivers tested but found negative for the infective stage.

The infection process is seasonal and is generally limited to about May to November (Schafer, 1968; Johnson, 1975; Udey et al., 1975; Johnson et al., 1979; Ratliff, 1983; Ching and Munday, 1984a). However, seasonal occurrence in California has been examined only at the Crystal Lake Hatchery in the Pit River drainage (Schafer, 1968). At Crystal Lake, infection did not occur at temperatures less than 10°C. January, February, and March were noninfective depending on water temperature. California rivers and streams with anadromous fish runs have not been studied in this regard. At warmer temperatures, the course of the disease is faster (Udey et al., 1975).

There is no known treatment for ceratomyxosis, hence prevention and management are paramount. Management involves stocking resistant species or strains wherever waters are known to harbor the infective stage and releasing hatchery fish during periods of low or no infectivity. The objectives of this study are to determine the geographic and seasonal occurrence of the infective stage of *C. shasta* in California and to determine relative susceptibilities of coho and chinook salmon strains to *C. shasta*. Such information is essential to management of resident and anadromous salmonids.

Geographic Distribution

Geographic distribution of the infective stage of *C. shasta* was determined by placing caged sentinels (Shasta strain rainbow trout) in test streams and rivers. When a river system was first tested, cages were placed near the mouth of the system but above tidal influence. Further exposures in the same system were done only if the infective stage was present and were always done further up river. Plastic minnow traps (43.5 cm long by 23 cm wide) with entrance holes plugged were used for field exposures. Up to 25 fish

Table 1. Geographic Distribution of the Infective Stage of *Ceratomyxa shasta* in California

Location and Watershed	Comments	Reference
Sacramento River	Upstream from mouth to Shasta Lake	Wolf and Manzer, unpublished (cited in Johnson et al., 1979)
Feather River	Downstream from Oroville to confluence with Sacramento River	Wolf and Manzer, unpublished (cited in Johnson et al., 1979)
Pit River	Between Sucker Springs and confluence with Fall River	Schafer, 1968
Shasta, Crystal, Baum, Rising River, and Britton Lakes		Schafer, 1968
Rising River		Schafer, 1968
Hat Creek (below Baum Lake)		Schafer, 1968
Fall River		Schafer, 1968
Klamath River	Upstream from mouth to and including Klamath Lake in Oregon	Johnson et al., 1979

Table 2. Sites Tested for Presence of Infective Stage of *Ceratomyxa shasta*, Map Locations, and Results

Test Site	Map Location	Result
Coastal Rivers		
Smith River	R1E, T17N, S32	Neg
Prairie Creek	R1E, T11N, S22	Neg
Redwood Creek	R1E, T10N, S11	Neg
Mad River	R2E, T6N, S31	Neg
Eel River	R1W, T3N, S29	Neg
Mattole River	R2W, T2S, S9	Neg
Ten-Mile River	R17W, T20N, S34	Neg
Noyo River	R17W, T18N, S1	Neg
Big River	R17W, T17N, S24	Neg
Gualala River	R15W, T11N, S26	Neg
Russian River	R11W, T7N, NE 1/4 sec (at Duncans Mills)	Neg
Navarro	R16W, T15N, S7	Neg
Klamath-Trinity System		
Klamath River at Klamath Glen	R2E, T13N, S19 (river mile 7)	Pos
Above Salmon River	R6E, T12N (river mile 71)	Pos
Above Scott River	R10W, T46N, S32 (river mile 144)	Pos
at Copco Lake	R4W, T48N, S35 (river mile 202)	Pos
Trinity River at Weitchpec	R4E, T9N, S10 (river mile 0)	Neg
at Willow Creek	R5E, T7N, S28 (river mile 25.5)	Neg
Salmon River	R6E, T11N, S2	Neg
Scott River	R10W, T45N, S21	Neg
Shasta River	R6W, T45N, S29	Neg
Bogus Creek	R5W, T47N, S13	Neg
Sacramento River System		
Sacramento River at Los Molinos	R2W, T25N (river mile 229)	Pos
Sacramento River just above Keswick Dam	R5W, T32N, S21 (river mile 302.5)	Pos
Sacramento River above Shasta Lake	R5W, T36N, S15	Neg
Feather River (between Yuba City/Marysville)	R3E, T15N (river mile 29)	Pos
Bear River	R5E, T13N, S2	Neg
Butte Creek	R1W, T16N, S35	Pos
San Joaquin River System		
North Mokelumne River	R4E, T4N (river mile 4)	Pos

(depending on size) were placed in minnow-trap cages. Caged sentinels were left in test streams for 10–14 days to allow infection to take place. Sentinels were not fed during exposure. Sentinels were then returned to the Humboldt State University (HSU) Telonicher Marine Laboratory for rearing.

Sentinels were reared for at least 70 days or until ceratomyxosis was diagnosed. This rearing period allowed development of the characteristic spores of *C. shasta*. Rearing was done at the HSU Telonicher Marine Laboratory. Individual lots of fish were kept separate at all times. Each lot was maintained in a self-contained rearing unit (tank and filter) to prevent cross-contamination. Sentinel fish were fed a maintenance ration throughout rearing. They were examined for ceratomyxosis either when they died or after 70 or more days of rearing. A small piece of lower intestine was removed from each fish, and two wet mounts were prepared from the fluid contents. Each wet mount was examined at 400x or 1000x for 10 min or until spores were observed. Diagnosis of ceratomyxosis was based on recovery of the characteristic kidney bean-shaped spores of *C. shasta*.

The infective stage of *C. shasta* was found in the main stem of the Klamath River; in the main stem of the Sacramento River; in some of the major tributaries to the Sacramento River, including the Feather River and Butte Creek; and in the North Mokelumne River (Table 2). Coastal rivers lack the infective stage.

Seasonal Occurrence

Field exposures to determine seasonal occurrence of the infective stage were carried out in the Klamath River at Klamath Glen, California during the winter of 1986–1987. This part of the river is highly infective. Twenty caged sentinels were placed in the river for 10 days at 10-day intervals. Minnow trap–type cages were also used for seasonal studies. Sentinels were not fed during exposure. River water temperature was monitored throughout the study. Sentinels were brought to the HSU Telonicher Marine Laboratory for rearing. Rearing of sentinels and methodology for diagnosing ceratomyxosis was the same as described for the study on geographic distribution.

In winter 1986, the Klamath River was infective up to and including the exposure interval December 5–15. At this time, water temperature was 7°C. The river was not infective until the spring of 1987. Sentinels were first infected during the exposure interval April 9–20, 1987, at a water temperature of 15°C.

Salmon Strain Susceptibility

Field exposures to determine relative susceptibilities of coho and chinook salmon strains were carried out in the Klamath River at Klamath Glen (north coast strains) or in the Sacramento River at Los Molinos (Sacramento River Valley strains). These parts of these rivers have been highly infective. In each case, Shasta rainbow trout were exposed concurrently to allow comparison between the two test sites. Thirty-five to 101 fish from each strain were marked by fin clipping and placed in their respective rivers for testing in 0.7-m^3 wood and plastic mesh traps. Caged fish were left in rivers for 3 days to allow infection to take place. Test strains were then taken to the HSU Fish Museum rearing tanks (north coast strains) or the California Department of Fish and Game Fish Disease Laboratory (Sacramento River Valley strains) for rearing. Test strains were randomly mixed and reared for 80–90 days at 15.5–17.5°C. Fish that died were collected daily for immediate examination or frozen for later examination. Fish surviving at 90 days were sacrificed and examined.

Methodology for diagnosing ceratomyxosis was the same as that described for the study of geographic distribution. Ceratomyxosis was considered to be the cause of death when characteristic signs of the disease were observed and when large numbers of spores were apparent in intestinal scrapings. Only fish that survived past the date when *C. shasta* spores could be identified were considered.

Four strains of coho salmon, one strain of chinook salmon, control Shasta rainbow trout, and a Shasta x Kamloops rainbow trout hybrid were exposed for 72 hours in the Klamath River (Table 3). Prairie Creek coho salmon had the highest percentage mortality (65.9%). Irongate fall chinook and Irongate coho had the lowest percentage mortality due to ceratomyxosis (6.1% and 6.7%, respectively). Mean time to death ranged from 25.2 days (Shasta x Kamloops rainbow trout) to 28.0 days (Irongate coho).

Four strains of chinook salmon were exposed with control rainbow trout for 72 hours in the Sacramento River at Los Molinos (Table 4). Initial deaths occurred from 27 to 34 days after exposure. The highest percentage mortality due to ceratomyxosis occurred in Nimbus fall chinook (14.3%); next were Feather River fall chinook (12.5%). Nimbus fall chinook also had the longest mean time to death (40.3 days). None of the 101 Shasta rainbow trout exposed with the four Sacramento River Valley chinook strains became infected.

Cooperating Organizations

California Department of Fish and Game
National Park Service
Prairie Creek County Fish Hatchery, Humboldt County
U. S. Fish and Wildlife Service
University of California, Davis

References

Buchanan, D. V., J. E. Sanders, J. L. Zinn, and J. L. Fryer. 1983. Relative susceptibility of four strains of summer steelhead to infection by *Ceratomyxa shasta*. *Trans. Am. Fish. Soc.* 112:541–543.

Ching, H. L. 1984. Comparative resistance of Oregon (Big Creek) and British Columbia (Capilano) juvenile chinook salmon to the myxozoan pathogen, *Ceratomyxa shasta*, after laboratory exposure to Fraser River water. *Can. J. Zool.* 62:1423–1424.

Ching, H. L., and D. R. Munday. 1984a. Geographic and seasonal distribution of the infectious stage of *Ceratomyxa shasta* Noble, 1950, a myxozoan salmonid pathogen in the Fraser River system. *Can. J. Zool.* 62:1075–1080.

Ching, H. L., and D. R. Munday. 1984b. Susceptibility of six Fraser chinook salmon stocks to *Ceratomyxa shasta* and the effects of salinity on ceratomyxosis. *Can. J. Zool.* 62:1081–1083.

Conrad, J. F., and M. Decew. 1966. First report of *Ceratomyxa* in juvenile salmonids in Oregon. *Prog. Fish-Cult.* 28:238.

Johnson, K. A. 1975. Host susceptibility, histopathologic, and transmission studies on *Ceratomxa shasta*, a myxosporidan parasite of salmonid fish. Doctoral dissertation, Oregon State University, Corvallis.

Johnson, K. A., J. E. Sanders, and J. L. Fryer. 1979. *Ceratomyxa shasta* in salmonids. Fish Diseases Leaflet No. 58. U.S. Fish and Wildlife Service, Washington, D.C.

Ratliff, D. E. 1981. *Ceratomyxa shasta*: Epizootiology in chinook salmon of

Table 3. Relative Susceptibilities of North Coast Chinook and Coho Salmon Strains to Ceratomyxosis

Strain	Number of Fish	Mortalities Due to Ceratomyxosis	Percentage Mortality	Days to First Death	Days to Last Death	Mean Time to Death (days)
Irongate fall chinook	49	3	6.1	24	26	25.7
Irongate coho	30	2	6.7	27	28	28.0
Prairie Creek coho	41	27	65.9	18	32	25.6
Noyo coho	39	10	25.6	20	28	25.6
Trinity coho	18	3	16.7	20	28	25.7
Shasta rainbow trout	32	13	40.6	21	32	25.4
Shasta x Kamloops rainbow trout	37	14	37.8	21	31	25.2

Note. Test fish were exposed in the Klamath River at Klamath Glen October 19–22, 1987.

Table 4. Relative Susceptibilities of Sacramento River Drainage Chinook Salmon Strains to Ceratomyxosis

Strain	Number of Fish	Mortalities Due to Ceratomyxosis	Percentage Mortality	Days to First Death	Days to Last Death	Mean Time to Death (days)
Nimbus fall chinook	21	3	14.3	34	45	40.3
Feather River spring chinook	64	2	3.1	32	36	34.5
Feather River fall chinook	48	6	12.5	27	33	30.5
Coleman fall chinook	50	2	4.0	32	33	33.0
Shasta rainbow trout	101	0	NA	NA	NA	NA

Note. Test fish were exposed in the Sacramento River at Los Molinos July 24–27, 1987. NA = not applicable.

central Oregon. *Trans. Am. Fish. Soc.* 110:507–513.
Ratliff, D. E. 1983. *Ceratomyxa shasta*: Longevity, distribution, timing and abundance of the infective stage in central Oregon. *Can. J. Fish. Aquat. Sci.* 40:1622–1632.
Rucker, R. R., B. J. Earp, and E. J. Ordal. 1954. Infectious diseases of Pacific salmon. *Trans. Am. Fish. Soc.* 83:297–312.
Sanders, J. E., J. L. Fryer, and R. W. Gould. 1970. Occurrence of the myxosporidan parasite *Ceratomyxa shasta*, in salmonid fish from the Columbia River basin and Oregon coastal streams. In *A Symposium on Diseases of Fishes and Shellfishes.* S. F. Snieszko, ed. Special Publ. No. 5. American Fisheries Society, Bethesda, Maryland. pp. 133–141
Sanders, J. E., J. L. Fryer, D. A. Leith, and K. D. Moore. 1972. Control of the infectious protozoan *Ceratomyxa shasta* by treating hatchery water supplies. *Prog. Fish-Cult.* 34:13–17.
Schafer, W. E. 1968. Studies on the epizootiology of the myxosporidan *Ceratomyxa shasta* Noble. *Calif. Fish Game* 54:90–99.
Udey, L. R., J. L. Fryer, and K. S. Pilcher. 1975. Relation of water temperature to ceratomyxosis in rainbow trout *(Salmo gairdneri)* and coho salmon *(Oncorhynchus kisutch)*. *J. Fish. Res. Board Can.* 32:1545–1551.
Wales, J. H., and H. Wolf. 1955. Three protozoan diseases of trout in California. *Calif. Fish Game* 41:183–187.
Zinn, J. L., K. A. Johnson, J. E. Sanders, and J. L. Fryer. 1977. Susceptibility of salmonid species and hatchery strains of chinook salmon *(Oncorhynchus tshawytscha)* to infections by *Ceratomyxa shasta*. *J. Fish. Res. Board Can.* 34:933–936.

Publications

Carleton, A. In prep. Geographic and seasonal distribution of the infective stage of *Ceratomyxa shasta* in northern California and comparative salmonid strain susceptibility. Master's Thesis, Humboldt State University, Arcata, California.
Hendrickson, G. L. and A. Carleton. 1988. Geographical and seasonal distribution of *Ceratomyxa shasta* (Myxozoa) in northern California. *Intl. Assoc. Aquat. Animal Med.* (IAAAM) *Proc.* 19:132–136.
Hendrickson, G. L., A. Carleton, and D. Manzer. In press. Geographic and seasonal distribution of the infective stage of *Ceratomyxa shasta* (Myxozoa) in northern California. In *Diseases of Aquatic Organisms.*

Lectures

Hendrickson, G. L. and A. Carleton. Geographic and seasonal distribution of *Ceratomyxa shasta* (Myxozoa) in northern California. International Association of Aquatic Animal Medicine, Orlando, Florida, May 1988.
Hendrickson, G. L. and A. Carleton. Ceratomyxosis in northern California. California Department of Fish and Game, Anadromous Hatchery Manager's Meeting, Arcata, California, September 1988.

Larval Feeding and Year-Class Strength of the Northern Anchovy *Engraulis mordax*

University of California San Diego
Scripps Institution of Oceanography
R/F-106
Project Initiated: October 1, 1985
Project Completed: March 31, 1988

Mark Huntley, Reuben Lasker, and Francis Haxo

What causes variability in the year-class strength of fishes? Hjort (1926) first suggested that year-class strength was determined by the quality and quantity of food available to first-feeding larvae. Lasker (1978) created renewed interest by proposing that this was applicable to northern anchovy, *Engraulis mordax*. Our goal is to test the hypothesis in detail.

Larval anchovy are thought to depend almost entirely on dinoflagellates for food during their first 10 days of life (Arthur, 1976; Scura and Jerde, 1977; Hunter, 1981). Many species of dinoflagellates occur in the coastal waters of California, and often form large blooms (Allen, 1941). However, recent evidence suggests that some of these may be of poor quality as food. This evidence comes from studies on pelagic copepods, zooplankton that share the same food resources used by northern anchovy. In copepods, dinoflagellates may inhibit feeding (Huntley, 1982; Huntley et al, 1983), cause regurgitation (Sykes and Huntley, in press), reduce survival (Huntley et al, 1985), delay development (Huntley et al. in press), or suppress reproduction (Huntley et al, 1985).

If dinoflagellates cause similar symptoms in larval anchovy, then we might expect them to influence the year-class strength of anchovy, as suggested by Hjort (1926).

Objectives

Our objectives were to quantify the relationship between larval anchovy feeding and year-class strength by addressing the following questions: (1) Is the feeding rate of first-feeding anchovy suppressed by certain species of dinoflagellates? (2) Are growth and survival suppressed on a diet of these dinoflagellates?

Methods

Larval *E. mordax* feed on particles >20 µm in diameter, and up to 200 µm in diameter (Scura and Jerde, 1977). Therefore, the dinoflagellates we tested were all >20 µm in diameter. The six species we tested, and their diameters, are shown in Table 1.

Table 1. Dinoflagellate Species Used in Experiments on Feeding, Growth, and Survival of Larval Northern Anchovy, *Engraulis mordax*

Species	Diameter (µm)
Gyrodinium dorsum	27
Gyrodinium resplendens	31
Gonyaulax catenella	33
Gonyaulax polyedra	40
Gonyaulax grindleyi	45
Gymnodinium splendens	48

Dinoflagellates were cultured in *Gonyaulax polyedra* medium (Loeblich, 1975). For our experiments, all cultures were used when they were in exponential growth phase.

Anchovy eggs were collected from gravid females and placed, in lots of 100, in 30 separate 15-l containers in a 17°C temperature-controlled laboratory. For each experiment, we used three treatments: (1) a dinoflagellate known to support growth (usually *Gymnodinium splendens*); (2) filtered seawater, which we expected to produce minimal survival and growth, and (3) the "test" dinoflagellate species.

For each treatment we used 10 containers with 100 larvae each. The containers with dinoflagellates were inolculated at levels of approximately 1 mg carbon/l, and were replenished daily to keep concentrations approximately constant. On each day of the experiment, larvae in one container from each treatment were sacrificed, their live standard lengths measured, their gut fullness noted, and their survival determined.

Our estimates of feeding rate were basid on the gut pigment method (Mackas and Bohrer, 1976). Eggs were procured from gravid females in the same manner as described for growth and survival experiments. Eggs were placed, in lots of 50, in 50 separate 5-l containers and allowed to hatch at 17°C. After they completely resorbed their yolk and had reached the first-feeding condition, the containers were inoculated with approximately 1 mg carbon/l of the test dinoflagellates. Then, at 1- to 2-hour intervals, the individuals in one container were sacrificed, separated into groups of 5 to 10 each, and placed in 5 ml of 100% methanol for the fluorometric determination of chlorophyll and chlorophyll-derived pigments contained in their guts.

Results

Results of the growth and survival experiments are shown in Table 2. For ease of comparison, we present the mean standard length on the last day of the experiment, both as an absolute value and as a percentage of the mean standard length attained in filtered seawater. In order, the growth was best on *Gymnodinium splendens* (177%), *Gyrodinium dorsum* (156%), and *Gyrodinium resplendens* (134%). Growth on *G. polyedra* was not significantly greater than in filtered seawater (117%), and growth on *Gonyaulax*

Table 2. Feeding, Growth, and Survival of Larval Northern Anchovy, *Engraulis mordax*, on Six Dinoflagellate Species as Compared with Filtered Seawater (FSW)

Species	Ingestion Rate (ng/hr)	Standard Length		Survival on Day 9
		mm	%	
Gyrodinium dorsum	0.04	4.55	156	0.13
Gyrodinium resplendens	0.12			
	0.12	3.94	134	0.60
Gonyaulax catenella	0.08			
	0.07			
Gonyaulax polyedra	0.18	3.43	117	0.05
Gonyaulax grindleyi	0.01			
	0.05	2.60	89	0.10
Gymnodinium splendens	0.13			
	0.16	5.16	177	0.85
Filtered seawater		2.92		0.19

Note. Feeding rates are expressed as nanograms of chlorophyll-equivalents per hour. Growth is inferred from the mean standard length of 9-day-old larvae and expressed as a percentage of the length attained in FSW. Survival of 9-day-old larvae is expressed as a fraction of the initial population.

grindleyi was worse (89%).

Survival followed more or less the same trend. On the last day of the experiments, survival in filtered seawater was only 19%. By contrast, it was 85% in *G. splendens* and 60% in *G. resplendens*. However, in the other treatments survival was not much different than in filtered seawater, being 13% in *G. dorsum*, 10% in *G. grindleyi* and only 5% in *G. polyedra*.

Feeding rates (Table 2) were greatest on *G. polyedra, G. splendens*, and *G. resplendens*. They were uniformly low on *Gonyaulax catenella, G. grindleyi*, and *G. dorsum*.

Conclusions

We find clear negative effects of several dinoflagellate species on the survival, growth, and feeding of larval *E. mordax*. Only two species, *G. splendens* and *G. resplendens*, gave uniformly positive results, yielding high rates of survival, feeding, and growth. *Gonyaulax grindleyi* yielded the greatest feeding rate, but yielded the poorest survival and growth. The other species we tested gave both poor feeding and survival rates.

The quality of dinoflagellates as food for larval anchovy is apparently not related to the size of the cell, nor to its carbon content. For example, both the best species (*G. splendens*) and one of the poorest (*G. grindleyi*) are approximately the same size (Table 1). We cannot say whether the quality of the food is due to the lack of essential nutrients or to the presence of some actively inhibitory substance(s). However, inhibitory substances in *G. grindleyi* are suggested by the fact that it yielded poorer growth and survival than filtered seawater.

What is the significance of our results to field populations of *E. mordax*? We think that year-class strength of northern anchovy (as well as other clupeoid fishes, which also depend on the same types of particles for food), may be strongly affected by the presence of noxious dinoflagellates. This supports the first-feeding hypothesis of Hjort (1926).

Cooperating Organizations

Southwest Fisheries Center, National Marine Fisheries Service, La Jolla

References

Allen, W. E. 1941. Twenty years' statistical studies of marine plankton dinoflagellates of Southern California. *Am. Midl. Nat.* 26:603–635.

Arthur, D. 1976. Food and feeding of larvae of three fishes occurring in the California Current, *Sardinops sagax, Engraulis mordax,* and *Trachyurus symmetricus*. *Fish. Bull.* 74:517–530.

Hjort, J. 1926. Fluctuations in the year classes of important food fishes. *J. Cons.* 1:5–38.

Hunter, J. R. 1981. Feeding ecology and predation of marine fish larvae. In *Marine Fish Larvae: Morphology, Ecology and Relation to Fisheries*. R. Lasker, ed. Washington Sea Grant Program, Seattle. pp. 34–37.

Huntley, M. 1982. Yellow water in La Jolla Bay, California, July 1980. II. Suppression of zoolplankton grazing. *J. Exp. Mar. Biol. Ecol.* 63:81–91.

Huntley, M., K.-G. Barthel, and J. L. Star. 1983. Particle rejection by *Calanus pacificus*: Discrimination between similarly sized particles. *Mar. Biol.* 74:151–160.

Huntley, M., P. Ciminiello, and M. D. G. Lopez. 1987. Importance of food quality in determining development and survival of *Calanus pacificus* (Copepoda: Calanoida). *Mar. Biol.* 95:103–113.

Huntley, M., P. Sykes, S. Rohan, and V. Marin. 1985. Chemically mediated rejection of dinoflagellate prey by the copepods *Calanus pacificus* and *Paracalanus parvus*: Mechanism, occurrence and significance. *Mar. Ecol. Prog. Ser.* 28:105–120.

Lasker, R. 1978. The relation between oceanographic conditions, and larval anchovy in the California Current: Identification of factors and contributions to recruitment failure. *Rapp. P.-v. Reun. Cons. Perm. Int. Explor. Mer* 173:212–230.

Loeblich, A. R., III. 1975. A seawater medium for dinoflagellates and the nutrition of *Cachonina niei*. *J. Phycol.* 11:80–86.

Mackas, D. L., and R. Bohrer. 1976. Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. *J. Exp. Mar. Biol. Ecol.* 25:77–85.

Scura, E., and C. W. Jerde. 1977. Various species of phytoplankton as food for larval northern anchovy, *Engraulis mordax*, and relative nutritional value of the dinoflagellates *Gymnodinium splendens* and *Gonyaulax polyedra*. *Fish. Bull.* 75:577–583.

Sykes, P., and M. Huntley. 1987. Acute physiological reactions of *Calanus pacificus* to selected dinoflagellates: Direct observations. *Mar. Biol.* 94:19–24.

Publications

Huntley, M. In press. Larval feeding of larval northern anchovy, *Engraulis mordax*, on dinoflagellates: Implications for year-class strength. *Topics in Marine Biology*, J. D. Ros, ed. *Scient. Mar.* 53.

Lectures

Hay, M. E., and W. Fenical. Integrating marine chemical ecology and biotechnology. A report to the National Science Foundation on the results of a workshop held at the Western Society of Naturalists Meeting, Hilo, Hawaii, December 1986 (participants representing M. Huntley: P. F. Sykes and M. Lopez).

Huntley, M. Feeding, growth and survival of larval northern anchovy, *Engraulis mordax*: Implications for year-class strength. Presented at the 22nd European Marine Biology Symposium, Barcelona, Spain, August 1987.

Huntley, M. Phytoplankton/zooplankton chemical interactions. Invited lecture at Western Society of Naturalists Meeting, Hilo, Hawaii, December 1986 (presented by Mai Lopez).

Estimation of Larval Fish Starvation Rates in the Sea with Application to Northern Anchovy Larvae

University of California, San Diego
Scripps Institution of Oceanography
R/F-107
Project Initiated: October 1, 1985
Project Completed: September 30, 1987

Andrew A. Benson

Primary Goal for First Year

The primary objective for 1985–86 was to calibrate laboratory-reared larvae with field animals growing at a normal growth rate, and from this data set to identify lipid criteria to be used for diagnosing the nutritional state of field-caught larvae. Our original laboratory rearing in 1984 was done under standard rearing conditions of abundant food (Lasker et al., 1970; Hunter, 1976). The assumption has been that these rearing conditions produce fish larvae that are representative of the field. None of the available techniques has been able to test this important assumption. We wanted to make sure that the criteria we developed in the laboratory, from analyzing starving groups of larvae, were indeed applicable to the field. Thus the laboratory animals should be reared under conditions that permit them to attain the biochemical composition and growth rate found in the sea.

Identification of Lipid Profiles in Field Larvae Growing at Normal Rates. To identify a base level of lipid profiles in the field, we analyzed the lipid content of anchovy larvae collected off Southern California from a habitat that was believed to be a good feeding ground for larvae. We found earlier that the otoliths can be read from defatted fish larvae, provided that after lipid extraction the larvae are rehydrated in a solution of ethanol + Tromethamine (TRIS). John Butler, at the Southwest Fisheries Center, aged 239 of the fish larvae from this field site after the lipid extraction. Figure 1 shows the age vs. standard length (SL) relationship for these larvae, which were growing at a normal rate. Figure 2 shows the cholesterol vs. SL relationship. The strong correlation ($r^2 = 0.86$) was not unexpected because cholesterol is a membrane constituent and thus an indicator of living weight. The triglyceride content of the larvae (storage energy) showed a considerably higher variation against SL (Figure 3). The polar lipid content (also a membrane constituent, but more labile than cholesterol during starvation) is shown in Figure 4 for the field site. A number of larvae >7 mm SL had very low polar lipid content, which suggests that the larvae were in a very poor state. In order to make a correct diagnosis, these fish larvae and others must be compared to larvae reared in the laboratory under a defined schedule of feeding and starvation.

Comparison with Larvae from Standard Laboratory Rearing. It became clear that the animals reared in the laboratory under standard rearing conditions had a much higher size-specific triglyceride contents than the field animals. The difference was large, on the order of two to four times higher than on the field station. Only a few larvae in the field had a triglyceride content as high as that for laboratory rearing. The membrane lipid contents of the larvae were similar between the laboratory and the field, although slightly higher for the older laboratory-reared larvae.

Figure 1.

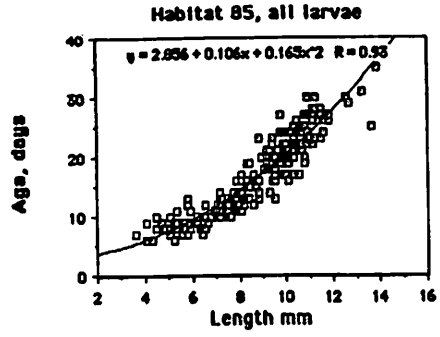


Figure 2.

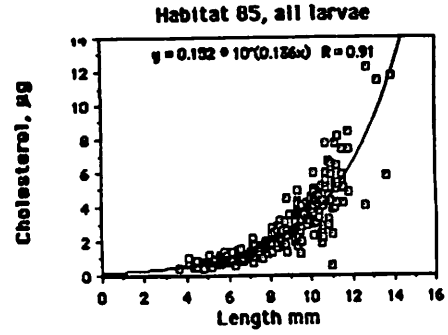


Figure 3.

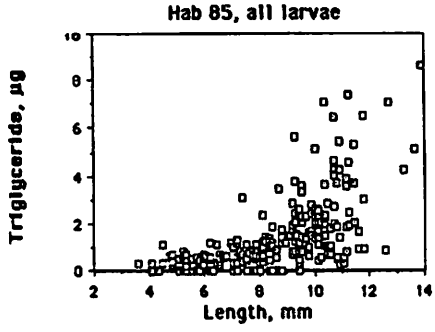
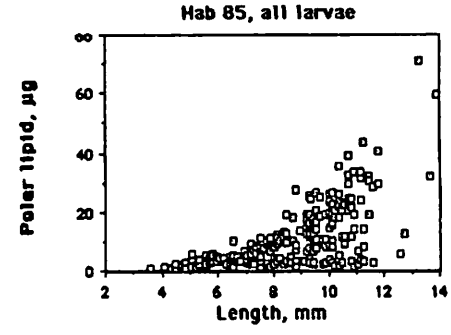


Figure 4.



Alterations in Feeding Regime to Provide Larvae Similar to the Field. To establish an appropriate feeding regime, we then reared five groups of anchovy larvae in substantially reduced food concentrations. In the standard rearing, 20–50 rotifers/ml *(Brachionus plicatus)* had been given the larvae, 15 of which were sampled every 2 days and analyzed individually for lipid content. In the new treatments, the food concentrations were lowered to between 0.2 and 3 rotifers/ml, and we also used the harpacticoid copepod *Tigriopus* as food in one treatment, for larvae older than 11 days. Twenty-five anchovy larvae were taken for lipid analysis from each treatment at ages 11 and 19 days. The results of these rearings are shown in Table 1, along with the values from the standard rearing for those 2 days. The dinoflagellate *Gymnodinium splendens* has often been used at a high concentration to provide a good (and presumably necessary) food source for first-feeding larvae (Lasker et al., 1970). We found no difference in any of the lipid components or in length at day 11 between larvae given *G. splendens* on day 4 plus rotifers on day 5, in comparison with larvae that were only given rotifers at day 5.

A good relationship was seen between the lipid content of the larvae and the amount of food offered (Table 1). This was true not only for triglyceride, but also for the membrane lipids. These results clearly indicate that the lipid components measured are good indicators of the condition of the fish. It was apparent that the mean lipid content was lower than in the ocean for treatments less than 1 rotifer/ml, whereas fish larvae could survive on a very low concentration of *Tigriopus* up to day 19 (<0.1/ml), although the lipid content was very small and the animals were somewhat undersized.

Results of Final Calibration Rearing. We used the preliminary rearings to set up the conditions for the calibration rearing. One rotifer per milliliter was given up to day 10. At day 10, *Tigriopus* copepodite stages were offered at 0.2/ml; the rotifers were kept at about 0.8/ml. Usually a small alga (*Isochrysis*) is added to the rearing containers as food for the rotifers (or copepods). This was not done in the calibration rearing with the aim of decreasing the triglyceride content of the food. In order to compensate for the expected higher variability in the fish larvae reared under a low food regimen, 35 larvae were removed from the rearing container every 2 days and analyzed for lipids. For the lipid components, these fish larvae were closer to those collected in the field samples.

Groups of fish larvae were removed from the rearing tank and starved over periods of 5 days. Thirty-five larvae were removed each day of starvation for analysis of individual lipid content. One group was starved from the egg stage, one group was starved from age 12 days, and one group from age 18 days. The triglyceride content on day 5 of starvation was only 20% to 30% of the day 1 values, for each of the starving groups. The membrane constituents were more stable, although they decreased during the later stages of starvation. The mean triglyceride/cholesterol ratio, one indicator of condition, decreased clearly for every day of starvation in each of the groups. Although the starving larvae decreased their lipid content in a reasonable fashion, we wanted to analyze more field animals before settling on the final criteria to be used for identifying starvation in the sea.

Table 1. Description of Food Treatments

1: Food concentration set at 0.2–1.0 rotifers/ml throughout rearing
2: Same as 1, except that dinoflagellates added initially
3: Same as 1, except that rotifers were kept without food
4: Food concentration cycled between 3 and 1 rotifer/ml throughout
5: 3 rotifers/ml up to day 11, then ~0.2 copepodites/ml
6: This was "standard" rearing, at 20–40 rotifers/ml

A. Samples taken on day 11 in each treatment

Treatment	Length	Triglyceride	Cholesterol	Polar Lipid
1	4.6 (0.1)	0.6 (0.1)	0.4 (0.04)	5.6 ((0.6)
2	4.6 (0.1)	0.6 (0.1)	0.4 (0.04)	6.6 (0.6)
3	4.8 (0.1)	0.5 (0.1)	0.3 (0.03)	6.0 (0.8)
4	5.1 (0.1)	0.6 (0.1)	0.4 (0.04)	6.2 (0.6)
5	5.9 (0.1)	1.2 (0.2)	0.7 (0.08)	not avail.
6	6.7 (0.2)	2.5 (0.3)	1.7 (0.02)	10.4 (1.3)

B. Samples taken on day 19 in each treatment

Treatment	Length	Triglyceride	Cholesterol	Polar Lipid
1	6.4 (0.2)	1.7 (0.3)	1.1 (0.2)	22.7 (4.1)
2	6.2 (0.2)	2.2 (0.4)	1.1 (0.2)	20.9 (4.0)
3	7.0 (0.2)	2.1 (0.4)	1.4 (0.2)	32.5 (4.8)
4	8.1 (0.2)	4.9 (0.6)	2.4 (0.2)	45.5 (5.3)
5	7.7 (0.1)	0.6 (0.1)	1.7 (0.1)	18.3 (2.2)
6	1.1 (0.3)	12.1 (1.9)	7.1 (0.7)	56.0 (7.2)

Note.—Lengths in mm; all lipids in µg; values in parentheses are standard error.

Primary Goal for Second Year

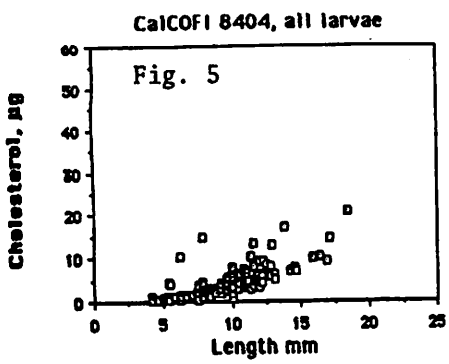
The primary objective for 1986–87 was to analyze a large number of fish larvae taken from frozen samples from each of the stations occupied by the CalCOFI cruises in the spring of 1986. More than 400 individual anchovy larvae were analyzed from each of these two cruises. In addition, we include the results of analyses performed on larvae obtained from the 8404 CalCOFI cruise. We were to use the measurements of larval condition to investigate whether there are differences between stations and whether ocean habitats that favor the growth and survival of anchovy larvae can be identified and described.

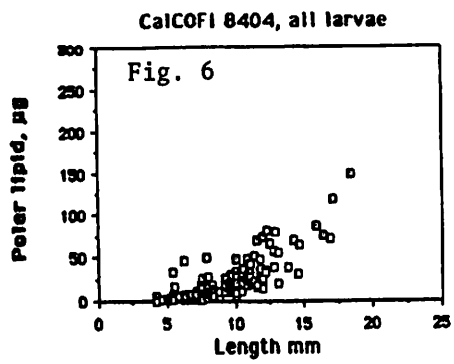
Results from CalCOFI cruises. The lipid components of all the larvae plotted against length for the 8404, 8602, and 8605 cruises are shown in Figures 5–13. The relationships are exponential as would be expected for the membrane constituents (cholesterol and polar lipid). Although the fit is good, there is still considerable variation in the lipid amount for any given length.

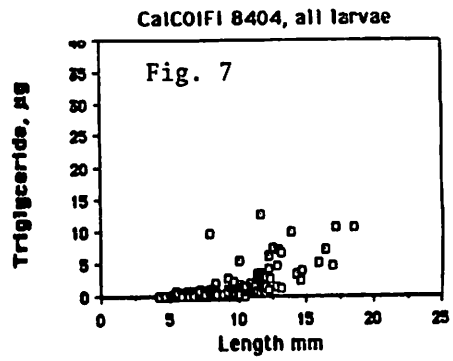
Number of larvae starving. From the results of the laboratory rearings, we set the criteria for larvae in moderate nutritional stress as larvae that had a very low triglyceride content (less than 30% of the cholesterol value) and a polar lipid that was less than 80% of the mean. For the 8602 cruise, we found that in the larval size category between 6 and 10 mm, 16% of the larvae fell into this category, collected on 14 of 22 stations. Also for the 8605 cruise, 16% of the larvae were in the group of nutritional stress, taken on 10 of 13 stations. For the 8404 cruise, as high as 28% fell into this category, and such larvae were found on all nine stations. From Figures 5–7 it can be seen that at least the mean triglyceride content of any size category fish is lower than for the 8602 and 8605 cruises.

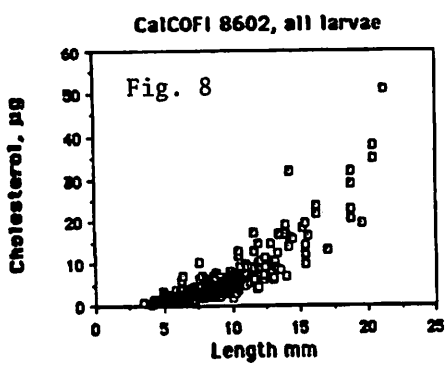
Test of Whether There is a Difference Between Stations. The first question to be asked was, is there a difference in the lipid components between the stations. A multivariate analysis of covariance was performed for each cruise by using the UCSD VAX/VMS system (BMDP program P4V.4): (1) The data were grouped by the stations (i.e., the between factor was station number). (2) Dependent variables were triglyceride, cholesterol, and polar lipid. (3) The covariate was length.

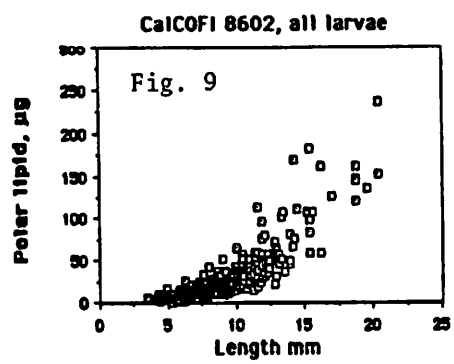
This run tested whether there was

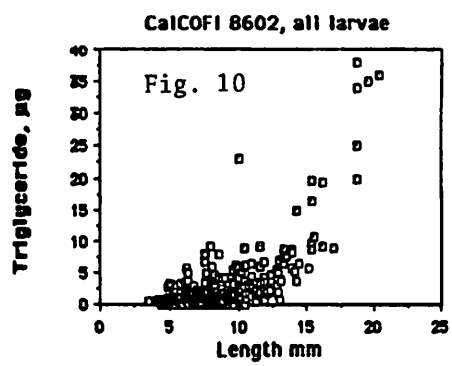


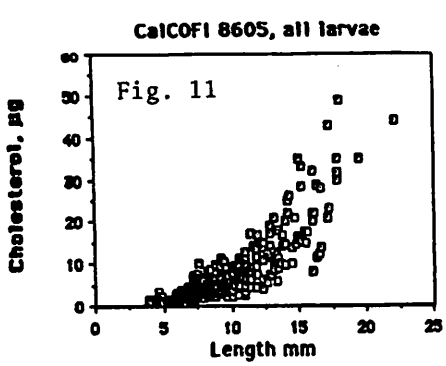


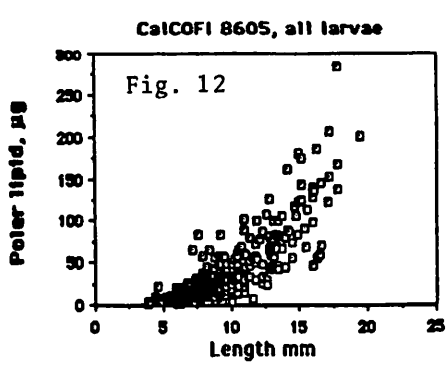


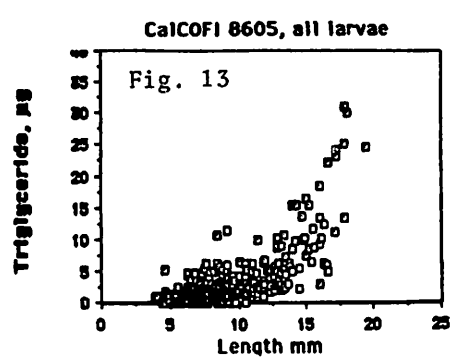












a significant difference in the lipid components between stations when the effect of length was taken out. The results showed that each of the lipid components was significantly different between the stations ($P < .001$) for each of the three cruises.

This result was encouraging as it showed that the ocean habitats do in fact differ in promoting the growth (and presumably survival) of anchovy larvae. It is especially noteworthy that the cholesterol content was also different between stations. We expected that the early larvae would all be growing at the same rate, and thus that the size-specific cholesterol content would be the same for all stations. In the first data set from the field, there was little difference in length-at-age (see Figure 1). However, these animals were taken in a fairly homogeneous habitat while following a drogue for 3 days. The significant difference in the cholesterol content means that the lipid-profile technique can be used to determine the condition of the larvae in general. Assessing the degree of starvation through the decrease in the more labile lipid components means investigating one aspect of "condition" only. The cholesterol component is indicative of the weight of the larvae, and we find it exciting that a difference was found. All the carcasses from the field study have been preserved in ethanol + TRIS, and it should be possible at some later date to get a measure of the weight of at least some of these larvae to test directly the relationship between cholesterol and weight.

Test of Whether the Larval Condition Can Be Related to Station Data. The second question to be asked was whether the condition of the larval anchovy can be correlated with variables measured on the CalCOFI stations: (1) Integrated chlorophyll *a*, (2) 10-m temperature, and (3) zooplankton volume. In addition to these variables, we had available to us measurements of the lipid content of the particle-grazing copepod *Calanus pacificus* from each occupied station. The wax ester content (changes over several days to a week) and the triglyceride content (changes over 1 to a few days) of the copepodite V stage of the copepods were used as additional variables to describe the station. These lipids in *Calanus* are good indicators of condition of the copepod (Hakanson, 1984, 1987). For the 8605 cruise, lipid measurements from one more particle-grazing copepod were added, the larger species *Rhincalanus nasutus*.

The tests performed were canonical correlation analyses. The anchovy larvae for each cruise were divided up into 1-mm size-groups (e.g., the 5-mm group, the 6-mm group). The relationship between the anchovy lipids and the station variables were then tested by canonical correlation analyses for each size-class. The results of these analyses (BMDP6M on the UCSD VAX/VMS) are shown in Table 2. The analysis tests whether

Table 2.

Cruise	Size-group of Larvae (mm)	No. of Stations	Significance in Bartlett's Test?	No. of Canonical Variables Needed	Highest Coefficients Canon. Var. (Anchovy)	Highest Coefficients Canon. Var. (Stations)
8602	5	8	Yes	1	Cholesterol	*Calanus* Triglyceride
	6	16	No			
	7	15	No			
	8	16	No			
	9	8	Yes	1	Cholesterol	10-m Temperature
	10	12	No			
8605	6	8	Yes	3	Cholesterol	*Rhincalanus* Triglyceride
	7	8	Yes	3	Cholesterol, Triglyceride	*Calanus* Triglyceride, 10-m Temperature
	8	11	Yes	1	Cholesterol	*Calanus* Triglyceride, 10-m Temperature
	9	10	Yes	1	Triglyceride	*Rhincalanus* Triglyceride
	10	12	Yes	1	Cholesterol, Triglyceride	*Rhincalanus* Triglyceride, *Calanus* Triglyceride
8404	6	6	Yes?($P = .062$)	1(?)	Cholesterol	Chlorophyll *a* *Calanus* Triglyceride
	7	7	Yes	2	Cholesterol	*Calanus* Triglyceride
	10	6	Yes	3	Triglyceride	*Calanus* Triglyceride, Chlorophyll *a*

the two sets of variables are dependent (Bartlett's test). Included in Table 2 is the number of variables needed to describe the dependence between the two sets of variables; those canonical variables that have the highest coefficients are given for each set.

Bartlett's test was significant for all the size-groups from the 8404 and 8605 cruises, but for only two of six size-groups from the 8602 cruise. These results are extremely encouraging because none of the station variables really were expected to be good indicators of the food environment for the anchovy larvae. They should be viewed with some caution, however, as the number of stations was fairly small in many cases. A relatively small change in a few stations might make a large difference. The lack of significance for the 8602 cruise may be due to seasonal effects of some kind, but the data available do not permit this to be tested.

The variable in the larval anchovy that was most important in the correlations was cholesterol. This is the most conservative component, and it presumably integrates over the longest time. Triglyceride seems important for the correlations most often for the larger larvae. At these sizes, the triglyceride content is higher, however, and thus in absolute terms this component would be integrating over a longer time.

The variables from the stations that usually were most important were the triglyceride measurements in the copepods. Triglycerides are the short-term energy store for the copepods and are an integrative measure of the organisms' grazing activity over the last few days.

Conclusions from the Project

1. Anchovy larvae can be reared in the laboratory to have a length-specific lipid content similar to that of field-caught larvae.
2. The food concentration needed in the laboratory to obtain this result was much lower than what has been used in a "standard" rearing.
3. We have confirmed that the lipid measurements are good indicators of the condition of the larvae.
4. The lipid analysis technique is reasonably rapid and can be used for analyzing large sets of individual fish from the field.
5. Standard net-haul procedures for sampling work well for the analysis, such as the 20-minute Bongo net tows used by CalCOFI. This is crucial when integrating the technique into a large-scale field program.
6. There were significant differences in the lipid components of the anchovy larvae between the stations for each of the three CalCOFI cruises that were investigated. This was the case also for the most conservative indicator of tissue weight, cholesterol. Thus, it is possible to use the lipid profiles for determining the condition of the larvae in general and not be limited to assessing degree of starvation in the larvae.
7. Canonical correlation analysis showed that the lipid components in the anchovy larvae were dependent on station data, at least for the two cruises undertaken in late spring.

Cooperating Organizations

California Cooperative Oceanic Fisheries Investigations
South West Fisheries Center, National Marine Fisheries Service

References

Håkanson, J. L. 1984. The long and short term feeding condition in field-caught *Calanus pacificus*, as determined from the lipid content. *Limnol. Oceanogr.* 29(4):794–804.

Håkanson, J. L. 1987. The feeding condition of *Calanus pacificus* and other zooplankton in relation to phytoplankton pigments in the California Current. *Limnol. Oceanogr.* 32(4):881–894.

Hunter, J. R. 1976. Culture and growth of northern anchovy, *Engraulis mordax*, larvae. *Fish. Bull. U.S.* 74:81–88.

Lasker, R., H. M. Feder, G. H. Theilacker, and May, R. C. 1970. Feeding, growth and survival of *Engraulis mordax* larvae reared in the laboratory. *Mar. Biol.* 5:345–353.

Publications

Håkanson, J. L. Lipid profiles for determining condition in anchovy larvae. Presented at CalCOFI meeting, Lake Arrowhead, California November, 1987.

Håkanson, J. L. 1989. Analysis of lipid components for determining the condition of anchovy larvae, *Engraulis mordax*. *Marine Biology* 102:143–151.

Håkanson, J. L. 1989. The condition of larval anchovy (*Engraulis mordax*) in the Southern California Bight, as measured through lipid analysis. *Marine Biology* 102:153–159.

Håkanson, J. L., and A. Benson. The use of lipid criteria to identify the condition of anchovy larvae in the sea. Manuscript.

Owen, R., J. Butler, N. Lo, G. H. Theilacker, A. Alvarino, J. Hunter, and J. L. Håkanson. Lagrangian assessment of well-being of larval anchovies in contrasting environments. Paper presented at CalCOFI meeting, Lake Arrowhead, California, October, 1986.

Lectures

Håkanson, J. L. Overview of techniques for determining the nutritional condition of copepods and fish larvae, with special emphasis on the use of lipid profiles in marine fish larvae. Invited paper presented at the American Society of Limnology and Oceanography Meeting, San Francisco, December 1986.

Håkanson, J. L. The condition of larval anchovy, *Engraulis mordax*, in the Southern California Bight, as measured through lipid analysis. Paper presented at the International Council for the Exploration of the Sea Symposium on the Early Life History of Fish Larvae, Bergen, Norway, October 3–5, 1988.

New Methods in Stock Abundance Estimation

Marc Mangel

University of California, Davis
R/F-109
Project Initiated: October 1, 1987
Project Completed: March 30, 1989

During the grant period, the following projects were investigated: (1) the relationship between catch per unit effort (CPUE) and stock abundance, (2) extensions of the methodology of Mangel and Beder (1985) for estimating abundance by using encounter rate data, (3) estimation of spawning stock abundance through the use of egg or larval survey data, and (4) estimation of movement variables for pelagic species.

It is commonly assumed in fisheries management that CPUE is proportional to stock abundance so that CPUE, which is an observable variable, is an indicator of abundance, which is not observable. Thus, if CPUE changes, it is often assumed that the stock level is changing in the same direction. There is increasing evidence, however, that such a proportional relationship is not always true. The first project involved a determination of the general relationship between CPUE and stock abundance. If N denotes the stock level, the general relationship is

$$CPUE = A N^p \qquad (1)$$

where the exponent p must be determined and A is a proportionality constant. If $p = 1$, then CPUE is propotional to N, but if $p < 1$, the phenomenon of "increasing catchability coefficient as stock size decreases" is observed. For the case in which $p < 1$, if a time series of CPUE shows a decline, then the true population level will have declined even more, and if CPUE oscillates, the true population will have oscillations of larger amplitude (bringing the population closer to a severe decline).

In Mangel (1986a, 1988), a variety of models relating CPUE to stock abundance are developed. These models involve explicit representation of the search process in fishing for schooled pelagic species and an operational model of the harvest process. In the simplest case, stock depletion is ignored and

$$CPUE = H(T)\, qN/(1 + T\, q\, N) \qquad (2)$$

where H(T) is the harvest from a set of time T once fish are encountered, and q is the search parameter. The search parameter is determined as follows: when the stock abundance level is N schools of fish, the time to detect a school is exponentially distributed with parameter qN.

Also in Mangel (1986a), the relationship between CPUE and stock abundance is studied for the case in which stock depletion occurs during the fishing process. A number of different models are developed based on different assumptions about search, harvest, and depletion. Each model shows three regimes: (1) CPUE independent of stock abundance, (2) CPUE directly proportional to stock abundance, and (3) CPUE given by Equation 1 with $p < 1$. A method for identifying the regime in which the operational parameters place the CPUE-stock abundance relationship is described. The method involves the use of maximum likelihood, given search time data. Mangel and Goulart (the trainee) have worked on extensions and testing the encounter rate methodology developed by Mangel and Beder (1985). This paper provides a means of estimating stock abundance by the use of search data and assumptions about the nature of encounter rates. The basic assumption is that if N schools were initially present and n have been captured, the time to the next encounter is exponentially distributed with parameter $q(N - n)$. Here q is the search parameter, computed from operational variables such as vessel speed v, detection width W, and area A in which the search operation occurs. In the simplest case, $q = Wv/A$. Mangel and Beder (1985) described both classical and Bayesian methods for estimating N, given encounter rate data, and show how such data can be used to estimate a probability distribution for N.

Mangel (1986b) has extended the methodology in a number of ways. These include (1) varying search capabilities of vessels, (2) the effects of stock aggregation on the estimate of N (the binomial model used by Mangel and Beder (1985) is replaced by a negative binomial model), (3) simultaneous estimation of the initial stock size and the search parameter, (4) learning by the fisherman (so that the area being searched is reduced over time—a common occurrence in many groundfish fisheries), and (5) explicit estimates of biomass. These methods are applied to a number of data sets, including Pacific Ocean perch and data on the Ivory Coast fishery for *Sardinella maderensis, Sardinella aurita, Scomber japonicus* and *Brachideuterus* provided by P. Cury (OSRAM, France) who was visiting the Pacific Environmental Group.

Goulart (1986, 1988) has extended the methodology of Mangel and Beder to migratory species. In such a situation, the simultaneous search in a number of different regions must be considered. Goulart developed appropriate theoretical estimates of initial stock size through the use of maximum likelihood estimates of abundance and applied the methodology to data on South Pacific tuna (mainly yellowfin and skipjack) collected by tagging vessels of the South Pacific Commission. Goulart's analysis of the data suggests that the actual search operation involves an essentially random search for

patches that contain a number of schools of tuna, followed by an exhaustive search within the patches. Thus, Goulart estimates the number of patches, the number of schools within a patch and the number of fish per school. These can be used to construct an estimate of biomass . Goulart tested the methodology using simulation techniques, so that the estimated measurements could be compared with the known true values. The results of the simulation study show the conditions under which the extended methodology works relatively well. Goulart also developed an "optimal search strategy" that allocates fishing effort to cells so that the maximal combination of fish and information is obtained.

This work led to an analysis of the migratory nature of pelagic species. In particular, we were approached by Dr. R. Hilborn of the South Pacific Commission for aid in the analysis of tagging data. The main difficulty with the analysis of tagging data is that recoveries involve uneven distributions of effort. Thus, imagine that at time $t = 0$, a number N_o of fish are tagged at the origin of a coordinate system. At some later time, tags are recovered in a small sector of the ocean where fishing happens to be occurring. That is, the ocean is not evenly sampled for fish. The key question is then: What can be inferred about the movement characteristics of the fish and abundance from recovered tag data?

Goulart's approach is to model the motion of the fish as a diffusion process in two dimensions. Thus, if $(X(t), Y(t))$ denotes the position of a certain fish at time t, we assume that the position at a later time $t + dt$ is normally distributed with mean $(X(t) + m_x dt, Y(t) + mdt)$ and variance s^2. The problem of inference is to tag fish, collect tagging data and then infer m_x, m_y, and s. The data consist of triplets (t_i, x_i, y_i), where the i^{th} fish was recaptured at time t_i at position (x_i, y_i). Goulart has developed methods based on likelihood analysis for the solution of this problem. He is able to predict maximum likelihood estimates for the parameters, Bayesian posterior distributions for the parameters, and the maximum *a posterior* estimates of the parameters. Of particular interest to us is the difference between the likelihood for the full paramter set in which all three parameters are estimated compared with a restricted parameter set in which we *a priori* set $m_x = m_y = 0$ (and thus assume that the fish only move randomly). Goulart has extended his methods to include natural and fishing mortality of the stock.

The final project was the development of methods that relate spawning stock levels to egg survey data. This work was done in close conjunction with P. Smith (Southwest Fisheries Center) and P. Wolf (California Department of Fish and Game) and was motivated by the current regulation and protection of Pacific sardine. Under current law, the sardine is protected from a direct fishery until the spawning biomass exceeds 20,000 short tons (at the turn of this century, the extimated spawning biomass exceeded 2 million tons), and agencies are mandated each year to determine whether the spawning biomass exceeds this level. It is also desirable to know if the biomass does exceed 20,000 tons, how much above the critical level it is because the stock in excess of the critical level can be fished, and quotas for the direct fishery must be determined.

Motivated by the work of Wolf and Smith (1985) on inverse egg production methods, Mangel (1987, 1988) developed a series of methods for estimating spawning biomass from egg survey data. The idea is that eggs are sampled at a discrete set of stations (the CalCOFI grid), and we wish to estimate the spawning biomass from discoveries of eggs and larva. The methods developed by Mangel involve presence-absence sampling. The number of eggs at the i^{th} station is assumed to be a random variable with a distribution involving two components. The first is the probability that a site is a habitat for the spawning stock in the current year. The second is the conditional distribution of eggs over sites, given that the site is a habitat. Because eggs are typically found in dense aggregations, Mangel assumes a contagious distribution such as the Neyman type A or the negative binomial. The critical spawning stock level is connected to the parameters of the contagious distribution. The model is used to predict the probability that a given site will have a positive number of eggs.

The field data consist of sampling a total of S stations, with S_p of these having eggs (positive stations) and S_n of these not having eggs (negative stations). We then estimate the probability that the mean of the underlying conditional distribution of eggs exceeds the critical value determined by the spawning biomass. The results (Mangel, 1987) have been encouraging and suggest that the methods have considerable versatility (e.g., the development of sequential sampling charts). Regarding this work, Dr. Rosenzweig of the University of Arizona has written, "The Mangel method promises a revolution in management techniques" (Rosenzweig 1987).

Cooperating Organizations

California Department of Fish and Game, Long Beach

Pacific Environmental Group, Monterey, California

South Pacific Commission, Noumea, New Caledonia

Southwest Fisheries Center, National Marine Fisheries Service, La Jolla, California

References

Goulart, J. A. 1986. An operational characterization of stock size estimation. Report 86-2. Department of Mathematics, University of California, Davis.

Goulart, J. A. 1988. Models for management of pelagic species. Doctoral dissertation, Applied Mathematics, University of California, Davis.

Mangel, M. 1986a. The relationship between catch per unit effort and stock abundance. Report 86-1. Department of Mathematics, University of California, Davis.
Mangel, M. 1986b. Abundance estimates by encounter rates. Report 86-4. Department of Mathematics, University of California, Davis.
Mangel, M. 1987. Sampling highly aggregated populations with application to California sardine management. *Lect. Notes in Biomath.* 2:247–263.
Mangel, M. 1988. In press. From egg surveys to ecosystem models: Biological assumptions in fisheries management. In *Biological Objectives in Fisheries Management.* W. Wooster, ed. Springer-Verlag, New York.
Mangel, M. and J. H. Beder. 1985. Search and stock depletion: Theory and applications. *Can. J. Fish. Aquat. Sci.* 42:150–163.
Rosenzweig, M. 1987. Comments on Mangel (1987). *Lect. Notes in Biomath.* 72:263.
Smith, P.E., and P. Wolf. 1985. An inverse egg production method for determining the relative magnitude of Pacific sardine spawning biomass off California. *CalCOFI Report* 26:130.

Publications

Goulart, J. A. 1986. An operational characterization of stock size estimation. Report 86-2. Department of Mathematics, University of California, Davis.
Mangel, M. 1986. Abundance estimates by encounter rates. Report 86-4. Department of Mathematics, University of California, Davis.
Mangel, M. 1986. The relationship between catch per unit effort and stock abundance. Report 86-1. Department of Mathematics, University of California, Davis.
Mangel, M. 1986. Sampling surveys for highly aggregated populations. Report 86-3. Department of Mathematics, University of California, Davis.
Mangel, M. 1987. Sampling highly aggregated populations with application to California sardine management. *Lect. Notes in Biomath.* 2:247–263.
Mangel, M. 1988. From egg surveys to ecosystem models: Biological assumptions in fisheries management. In *Biological Objectives in Fisheries Management.* W. Wooster, ed. Springer-Verlag, New York.
Mangel, M. and C. W. Clark. 1986. Search theory in natural resource modelling. *Natural Resource Modeling* 1:1–54.

Lectures

Goulart, J. Stock estimation of migratory species: An operational approach. Marine Fisheries Section, American Fisheries Society Meeting, Sacramento, California, February 1987.
Goulart, J. On the migratory nature of tuna. 7th Pacific Coast Resource Modeling Conference, Vancouver, Canada, June 1987.
Goulart, J. Estimating drift and diffusion parameters for migratory species. Week of Fisheries, Azores, November 1987.
Mangel, M. Statistical and computer methods for fisheries analysis. California Department of Fish and Game, Long Beach, October 1985.
Mangel, M. Sampling highly aggregated populations. Second workshop on Control Theory in Renewable Resource Management, Honolulu, December 1985.
Mangel, M. Issues and perhaps answers in plankton sampling. 5th Pacific Coast Resource Modeling Conference, Newport, Oregon, June 1986.
Mangel, M. From egg surveys to ecosystem models. Workshop on Biological Objectives in Fisheries Management, Seattle, Washington, January 1987.
Mangel, J. Models for the abundance of sardine. 7th Pacific Coast Resource Modeling Conference, Vancouver, B.C., Canada, June 1987.

The Effects of Freezing and Frozen Storage on the Status of Fish Tissue

University of California, Davis
R/F-110
Project Initiated: October 1, 1985
Project Completed: September 30, 1987

David S. Reid

In common with the earlier years of the project, during this final year, several batches of rockfish have been processed. These fish have been caught especially for the project and have been transported immediately to Davis on ice. On arrival at Davis, the fish have been prepared for freezing and have been frozen either rapidly or slowly. Individual fish have then been stored at one of three storage temperatures: –5°C, –12°C, or –20°C. Before freezing, material has been analyzed to obtain a chemical and biochemical characterization of the fresh fish. Small samples of the fish have been fixed to allow microscopic characterization of the fresh material. Samples taken immediately after freezing have also been assayed. The changes that take place during extended frozen storage can then be followed. Individual fish have been carefully identified throughout this study, as variation between individuals can be significant. Separate batches of fish have contained a variety of species of rockfish, which has allowed us to determine some of the differences in their responses to freezing.

To follow the changes that take place in the structure of the frozen fish and to assess the structural damage consequent upon the formation of ice within the tissue, we use a special fixation method that makes the fish tissue suitable for further optical or electron microscopic examination without disturbing the ice structures within the tissue. This specialized technique, known as isothermal freeze fixation (Asquith and Reid, 1980), was first applied to fish in an earlier rapid-response project and has been further developed in this study (Lampila et al., 1985). Thermodynamic principles are used to ensure that the fixative does not perturb the ice present within the tissue.

Different microscopic techniques are appropriate to investigate different aspects of the frozen tissue. Optical microscopy allows us to visualize the ice crystals within the frozen tissue easily, and we are therefore able to determine the amount, size, number, and location of the crystals. We have built up a set of slides for optical microscopy that show the changes in ice crystal character as a function of freezing method, storage temperature, and storage time. Inspection clearly shows the effect of storage temperature on the amount of ice within the tissue. It also shows the changes that take place in ice crystal size with time of storage. Quantification of the photomicrographs through the methods of image analysis clearly shows the increase in the size of ice crystals with time. The rate increases at higher temperatures of storage. The initial size of ice crystals also depends on the freezing method. It also appears that the kinetics of the change in the size of ice crystals on storage may be affected by the freezing method. The species of rockfish has no noticeable effect on the frozen structure.

Scanning electron microscopy (SEM) gives us a three-dimensional view of the frozen tissue and clearly shows the ice damage. The qualitative observations that can be made from the SEM photomicrographs of fish tissues frozen and stored under different conditions are in accord with the observations made by using the optical photomicrographs, (Doong, 1987). Transmission electron microscopy (TEM) allows us to visualize the changes that take place in the matrix between ice crystals consequent on ice formation. The matrix is quite compacted because of the dehydrating effect of ice formation. Conventional structures are difficult to discern. Examination of tissue thawed before fixation shows many of the structural features again, showing that the myofibrils relax back. Organelles, however, tend to be destroyed. The increasing concentration of the matrix at the lower temperatures of storage is readily apparent.

We have reported preliminary quantitative analysis of the microscopic data at a meeting on fish quality (Reid et al., 1986) and are continuing with data analysis. There is much still to be learned from the photomicrographs. We are attempting objective quantitative analysis rather than qualitative comparisons between pictures. The results suggest some interesting effects may be visible.

The microstructural data, in particular those data relating to tissue damage and complexity of continuous pathways between ice crystals within the matrix are analyzed also in the light of data generated on the chemical and biochemical changes taking place in the fish during storage. As attempts to miniaturize assays have not been entirely successful, we have been unable to perform a full range of studies on an individual fish. Sampling variation dictates a minimal sample size of around 10 g. We therefore follow particular chemical and biochemical indicators on individual fish rather than following all indicators on each fish. As expected, protein solubilities decrease with extended storage. The rate of decrease is more rapid at the higher temperatures. Adenosine triphosphatase activity of

the myosin also decreases during frozen storage. Again, the rate is faster at the higher temperatures. In both cases, an effect of freezing rate is also seen, but this effect is secondary to the effect of storage temperature. The pH of tissue did not change significantly during storage, but the tissue exhibited water loss. This was more marked for slowly frozen tissue and increased more rapidly at higher temperatures of storage.

The technique of differential scanning calorimetry has also been used to examine changes in the proteins in the intact, frozen fish tissue. An extraction method is not required. The results show a slow loss in native character of the proteins, (i.e., the size of the denaturation peak on a heating scan decreases). This effect is slow but is most rapid with storage at –5°C. We have obtained data for several species of rockfish and are in the process of collecting data relating to longer periods of storage.

Extracted lipids from the stored fish exhibit increased oxidative changes. Characterization has not been entirely successful. We are in the process of analysing a new set of samples and hope to complete the assessment in the near future. The data as yet are inconclusive.

Cryomicroscopy, which allows us to observe freezing as it happens, has been used to observe the freezing of isolated segments of fish muscle. The data indicate that internal freezing within the muscle occurs early in the freezing process. The membranes provide little resistance to ice propagation. We have analyzed the temperature profiles that exist within fish samples during a freezing process. This was done to estimate the temperature history at those points at which microscopy samples were taken, to deterime the importance of temperature history in a location to the frozen structure at the same location. The data do not as yet yield a clear picture. We added further chemical and biochemical methods of characterization for the later batches of fish. We measured the production of hypoxanthine, which is often equated with loss of quality of the fish. The data on hypoxanthine formation indicate that quality is lost rapidly at –5°C storage and more slowly at –20°C. The thiobarbituric acid (TBA) test can be used to estimate malondialdehyde levels, which are presumed to result from lipid oxidation processes. Species-dependent differences may exist. More data are needed to confirm this tentative observation. Once again, temperature of storage has a clear effect, with the most rapid change occurring at –5°C. The initial freezing rate also appears to have a clear effect at –5°C, with the slowly frozen material exhibiting more rapid malondialdehyde production. At extended times the TBA value falls. Such behavior has been ascribed to the formation of aggregated complexes with the proteins and is expected to produce detrimental textural effects.

We still have some data collection to perform on samples processed just before the completion date of the project, which will be done. We are well into the final phase of data analysis.

Cooperating Organizations
American Frozen Food Institute

References

Asquith, M. H. and Reid, D. S. 1980. The preparation of frozen samples for electron and light microscopy by isothermal freeze fixation at temperatures down to -20°C. *Cryoletters* 1:352–359.

Doong, N. F. 1987. The effects of freezing and frozen storage on the status of fish tissue. Doctoral thesis, University of California, Davis.

Lampila, L. E., V. Mohr, and D. S. Reid. 1985. Scanning electron microscopic study of rockfish preserved at either ambient temperature or by isothermal freeze fixation. *Food Microstruct.* 4:11-16.

Reid, D. S., N. F. Doong, M. Snider, and A. Foin. 1986. Changes in the quality and microstructure of frozen rockfish. Paper presented at the International Fish Quality Symposium, Anchorage, Alaska, November 1986.

Publications

Doong, N. F. 1987. The effects of freezing and frozen storage on the status of fish tissue. Doctoral thesis, University of California, Davis.

Reid, D. S. Quality of frozen rockfish. Paper presented at Pacific Fisheries Technologists meeting, Monterey, California, 1987.

Reid, D. S., N. F. Doong, A. Foin, and M. Snider. 1985. Studies on the frozen storage of fish. In *Storage lives of chilled and frozen fish and fish products.* IIR 1985, annex 4. pp. 111–118.

Reid, D. S., N. F. Doong, M. Snider, and A. Foin. 1987. Changes in the quality and microstructure of frozen rockfish. In *Seafood Quality Determination: Development in Food Science #15.* D. Kramer and J. Liston, eds. Elsevier Science Publishers, Amsterdam. pp. 1–14.

Correlation Between the Whiting Fishery and The Biomass of Whiting Food

Scripps Institution of Oceanography,
University of California, San Diego
R/F-111
Project Initiated: October 1, 1986
Project Completed: September 30, 1988

Michael M. Mullin

The original objectives of this project were to aid in the management of the whiting fishery by determining whether changes in fishery pressure have resulted in an increase in food for whiting and, therefore, perhaps changed the growth rate of whiting. The specific objective was to determine whether the biomass of euphausiids in the northern California area of the fishery was different after initiation of the fishery (1966) than before, following adjustment for biomass changes due to other causes. The rationale was that generally this research should add evidence for or against the necessity of a "multispecies" or "ecosystem" approach to fisheries management. Specifically, the managers of the joint-venture whiting fishery should know the degree to which growth dynamics of whiting have changed as a result of the fishery's indirect effect on whiting food.

The availability of zooplankton samples taken since 1949 from the California Current by the California Cooperative Oceanic Fisheries Investigation permitted us to attempt to detect an increase in the biomass of a prey of Pacific whiting, euphausiids, after initiation of a foreign fishery for whiting in 1966 off the coasts of Washington, Oregon, and northern California. Much evidence from freshwater lakes, and from the marine intertidal, indicates that the composition of prey species can change drastically after a major predator is removed or added to the environment. Evidence from catches of commercial shrimp off northern California and Oregon, which increased after l966, suggested that removal of whiting by the fishery might have a similar effect as large whiting eat shrimp. Euphausiids (smaller, shrimp-like crustaceans) are a major prey of whiting, especially fish <45 cm long, and are caught in nocturnal zooplankton tows. We therefore determined the dry-weight biomass of euphausiids in suoh tows off central and northern California through the 1960s.

Biomass of zooplankton changes from nearshore to offshore, from north to south, and interannually in the California Current. This means that the biomass of euphausiids could have changed concurrently with the initiation of the whiting fishery, but for other reasons. Zooplankters smaller than euphausiids are not eaten significantly by postlarval whiting but might be affected similarly by environmental variability. We therefore determined the dry-weight biomass of small zooplankton (separated by sieving) in the zooplankton samples to see if the *ratio* of euphausiid to small-zooplankton biomass changed, assuming that the fishery could cause an increase in this ratio but environmental variability might not. We tested this assumption by using published data to determine such ratios for the 1950s, when a major El Niño perturbed the California Current. Finally, we compared any changes in biomass off central and northern California, near the region of the whiting fishery, to change off southern and Baja California where there was no fishery. This was done to account for large-scale environmental changes, affecting both north and south, other than the fishery for whiting. We also used published data in an analogous way to test for an effect of the fishery on euphausiid biomass off Oregon.

Data from the 1950s confirmed that onshore-offshore, north-south, and interannual patterns of euphausiid and small-zooplankton biomasses were similar and that the ratio of biomasses was insensitive to environmental variability of these sorts. These results supported our use of small-zooplankton biomass to "correct" for changes in euphausiid biomass not related to the whiting fishery.

We were unable to detect, in either the data we collected from the California Current or in the data from Oregon, an increase in the biomass of euphausiid or in the euphausiid/small zooplankton ratio in 1966–1969 relative to 1960–1965. We were therefore unable to reject the null hypothesis that the removal of whiting by the fishery had no effect on the biomass of euphausiids.

Given this result, we had no reason to suppose that the food supply for whiting increased after the start of the fishery, so we did not attempt a calculation. Rather, we used a published bioenergetic model of the virgin and fished whiting populations to calculate the biomass of euphausiids that might accumulate for 2 years after the start of the fishery (i.e., the euphausiids the caught whiting would have eaten had they not been caught). We then used the measured variability of euphausiid biomass within the area of the fishery to calculate how many samples would have been needed, given the observed variability, to detect this accumulation at a statistically significant level. This calculation, though much idealized, indicated that, at minimum, three times as many samples would have been needed as were available. That is, natural variability was so great that many more samples than were available would have been needed to detect the increase in euphausiid biomass.

We think this project has three significant facets: (1) An environmental perturbation on the

scale of the El Niño of 1958–59 has much more significant effects on the biomass of whiting prey (euphausiids) than did the removal of part of the whiting stock by fishing. This could be either because the zooplanktonic community adjusts rapidly to changes in predation pressure from fish (the fish stocks vary naturally as well as because of fishing) or because predation by whiting contributes little to the limitation of euphausiid biomass and therefore exercises no control. (2) Several categories of zooplankton show similar patterns of geographical and interannual variation, and it is therefore possible to use changes in one category to separate general environmental causes from category-specific causes for change in another category. (3) If the variability in zooplankton samples taken with specified gear in a certain area is known, and an estimate of an expected change or difference between two groups of such samples is available, it is possible (and desirable) to estimate the numbers of samples from each group that must be analyzed if the expected difference is to be detected as statistically significant.

Cooperating Organizations

National Marine Fisheries Service, Northwest Fisheries Center, Seattle, Washington

National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, California

Oregon State University

Publications

Mullin, M. M. and A. Conversi. In press. Biomasses of euphausiids and smaller zooplankton in the California Current: Geographic and interannual comparisons relative to the Pacific whiting fishery. Fish. Bull.

Lectures

Conversi, A., and M. M. Mullin. Influence of the El Niño 1958 and the Pacific whiting fishery of 1966 on eyphausiid and copepod biomasses. Presented at the CalCOFI meeting, November 1988.

Mullin, M. M., and A. Conversi. Biomasses of euphausiids and smaller zooplankton in the California Current: Geographic and interannual comparisons relative to the Pacific whiting fishery. Presented at the winter meeting of the American Geophysical Union/American Society of Limnology and Oceanography, December, 1988.

Biochemical Indices of Metabolism and Growth in the California Halibut

University of California, San Diego
R/F-116
Project Initiated: October 1, 1987
Project Completed: September 30, 1988

George N. Somero

A primary objective in our Sea Grant–funded research with the California halibut and other species has been the development of biochemical indices of the organisms' physiological state. The development of biochemical indicators of the physiological state of laboratory fish maintained under carefully controlled conditions will lead to an ability to gauge the state of field-caught fish that have developed under generally unknown environmental conditions (e.g., of diet and activity). What must be demonstrated in these attempts is that a particular tissue and a particular set of biochemical measurements can be accurate indicators of the physiological state of the intact organism, as this state is influenced by such variables as exercise, diet, and ontogenetic stage.

Previously, we showed that the activities of certain enzymes of adenosine triphosphate–generating pathways of locomotory muscle, notably lactate dehydrogenase (LDH) of glycolysis and citrate synthase (CS) of the citric acid cycle, provide quantitative estimates of the muscle's capacity for peak energy production and "basal" metabolism, respectively. Because locomotory muscle, specifically white muscle, makes up the major share of body mass and accounts for a large fraction of the total metabolism of a fish, we have been able to develop our biochemical indices very satisfactorily by using

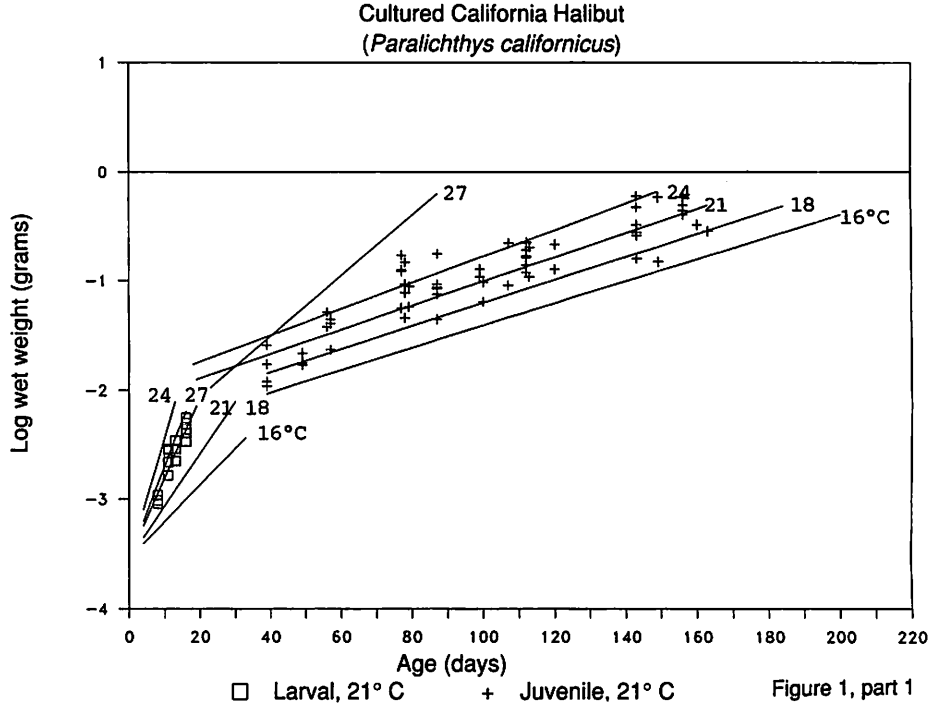


Figure 1, part 1

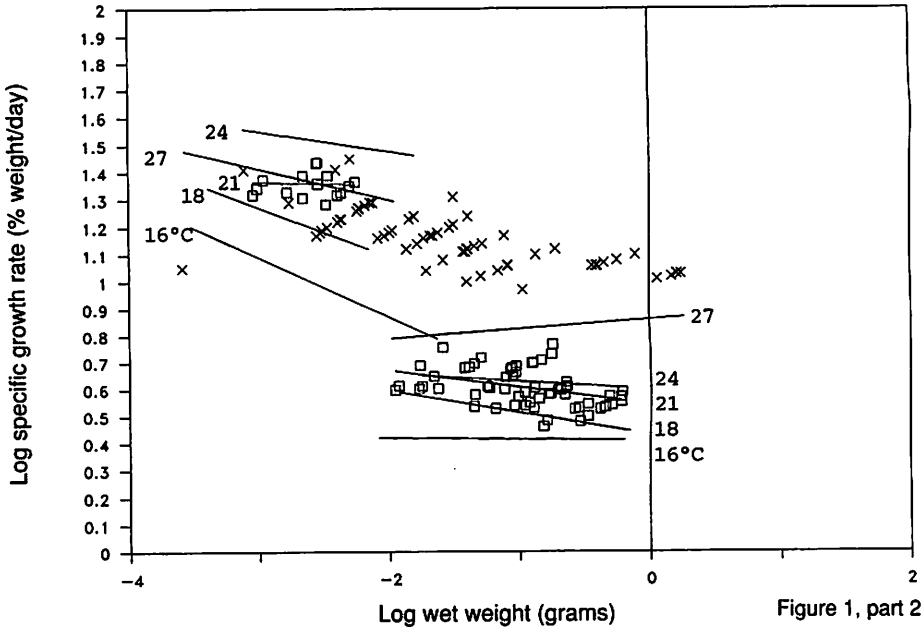


Figure 1, part 2

Figure 1. Growth of larval and juvenile California halibut at 16, 18, 21, 24, and 27°C. Live weight as a function of age is displayed for five rearing temperatures, and data is shown for fish reared at 21°C in the top figure. The specific growth rate as a function of weight is displayed in the lower graph. Data is shown for halibut and sardines reared at 21° C, and regression lines are displayed for all five rearing temperatures. Notice that the regression of growth rate against weight is divided at the larval to juvenile transformation weight (0.001 g).

this one tissue. In addition to the enzymatic indices of metabolic capacity, the ratio of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA), the RNA/DNA ratio, has been shown by several workers, including us, to provide a strong index of the current growth potential of the organism. Using these two indices, we have conducted a variety of laboratory acclimation experiments in which strong relationships between holding conditions and biochemical properties were developed.

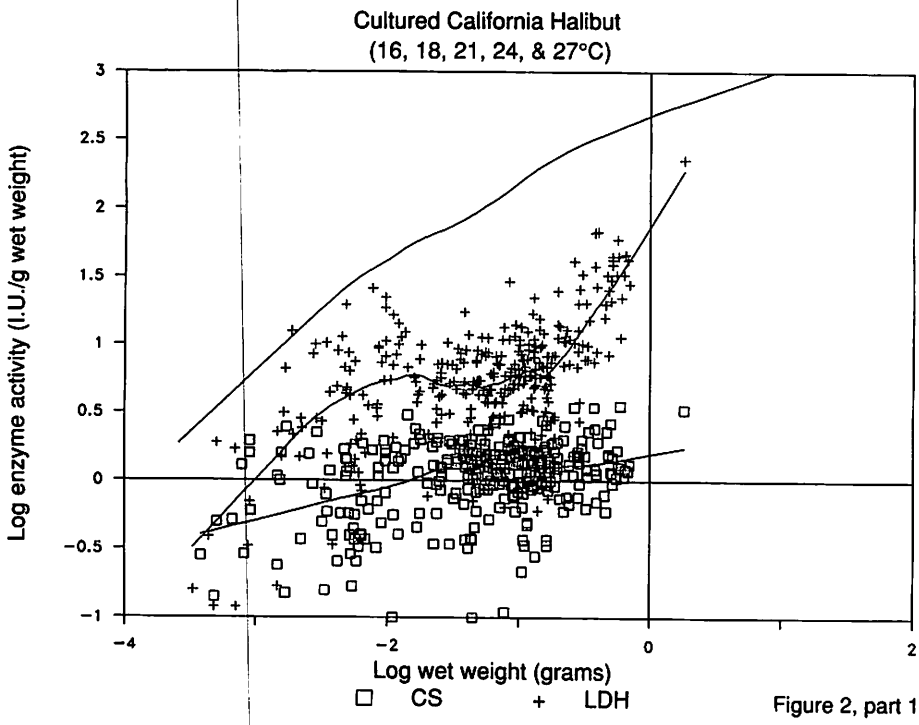


Figure 2, part 1

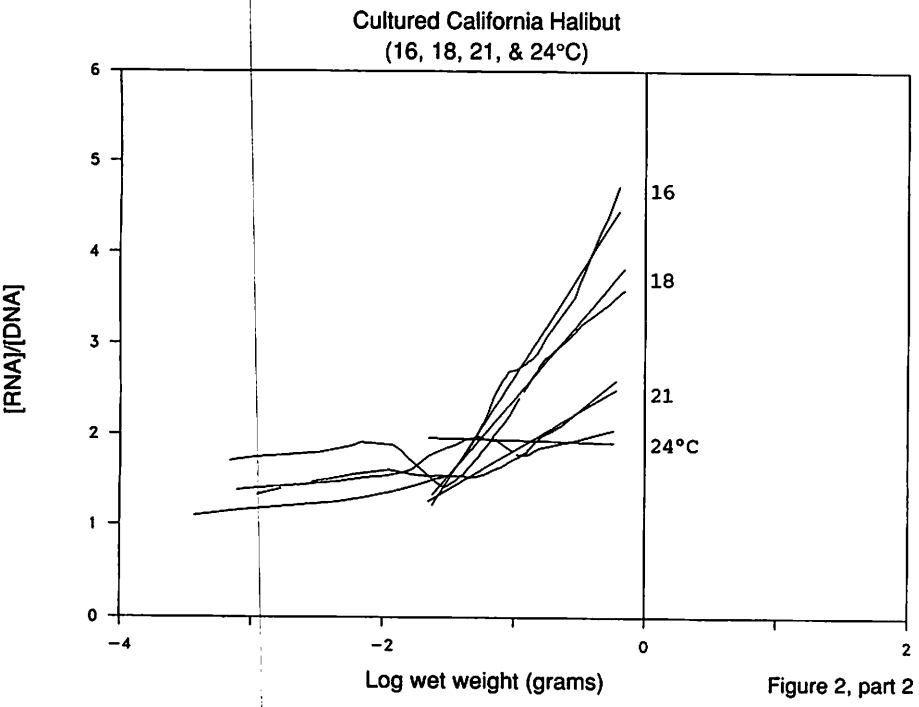


Figure 2, part 2

Figure 2. The top graph depicts the weight-specific activity of LDH and CS in the white trunk muscles of larval and juvenile California halibut as a function of the live weight of the fish (data from halibut at all rearing temperatures). The lower graph details the average ratio of the concentration of RNA to DNA for some of the same fish. Notice that both measures show a reduction in slope in the region of 0.03 to 0.04 g (log = −1.5 to −1.4).

Our studies of larval and juvenile California halibut (*Paralichthys californicus*) were initiated for several reasons. First, like many flatfishes, the California halibut undergoes a complex series of ontogenetic changes (e.g., transitions from an actively swimming, pelagic larva to a demersal juvenile). Second, in our earlier work we observed that a pronounced decrease in enzymatic activity and RNA/DNA ratio occurred very early in the juvenile phase. We wished to determine what the consequences of this developmental rate change were in terms of growth rate and metabolism. Third, the early ontogeny of the California halibut involves exposure of individuals to a wide range of habitat temperatures. Individuals may be found in cool, coastal waters (15–16°C) and in warmer bays and estuaries (often >20°C). We were interested in determining how the full range of environmental temperatures experienced by larval and juvenile California halibut affect the rate of growth, metabolism, and our biochemical indicators, and we wished to correlate our newly derived indices with well-established measures of growth. Fourth, an extensive field survey of California halibut by J. R. Hunter and S. Kramer of Scripps Institution of Oceanography and the Southwest Fisheries Center ensured us an adequate supply of field-caught larvae and juveniles, which could be compared with our laboratory-reared fishes. Last, we wished to develop an entirely new type of biochemical indicator: a means for estimating scope for metabolism of an individual fish by use of enzymatic analyses. An estimate of metabolic scope provides an excellent indicator of the fish's capacity for elevating its metabolic rate, and

thereby, capacity for growth.

Our experiments entailed the following major tasks. First, California halibut were grown from eggs at a series of temperatures that simulated the environmental temperature range of the species in Southern California waters (16, 18, 21, 24, and 27°C) Second, rates of growth and metabolism (oxygen consumption) were measured for individual fishes. Third, these same fishes were quick-frozen and their trunk musculature analyzed for LDH and CS activities and RNA/DNA ratio. Therefore, for the same specimens, we were able to correlate whole-fish characteristics (growth rate, respiration rate under postfeeding and postabsorptive conditions) with biochemical properties of white trunk muscles.

Our results reveal the following relationships. First, temperature has a profound effect on the growth rate and the pattern of growth (Figure 1). As temperature increased, the rate of growth exhibited the expected exponential rise for a fish of a given weight. Interestingly, the growth rate response to temperature changed during ontogeny. Thus, at the highest growth temperature, 27°C, the dip in growth rate (and biochemical indicators; see following) after the larval to juvenile transformation was less dramatic (about twofold) than at 16°C, the lowest temperature tested (about fivefold). The growth-rate data display distinct changes at most temperatures, and these changes are reflected distinctly in the biochemical indicators (i.e., the enzymatic and nucleic acid indices) (Figure 2).

Our attempt to develop an enzymatic index of metabolic scope was successful. We reasoned that the activity of CS could provide a quantitative estimate of the resting metabolic rate, whereas LDH activity would reflect the maximal metabolic rate of which the fish was capable. Thus, the difference between LDH and CS activity in locomotory muscle could serve as an index of metabolic scope. We induced a very high oxygen consumption rate (near maximal, see Priede, 1985) by feeding the fish to satiation. This high oxygen consumption rate was a consequence of very high food processing, absorption, and growth

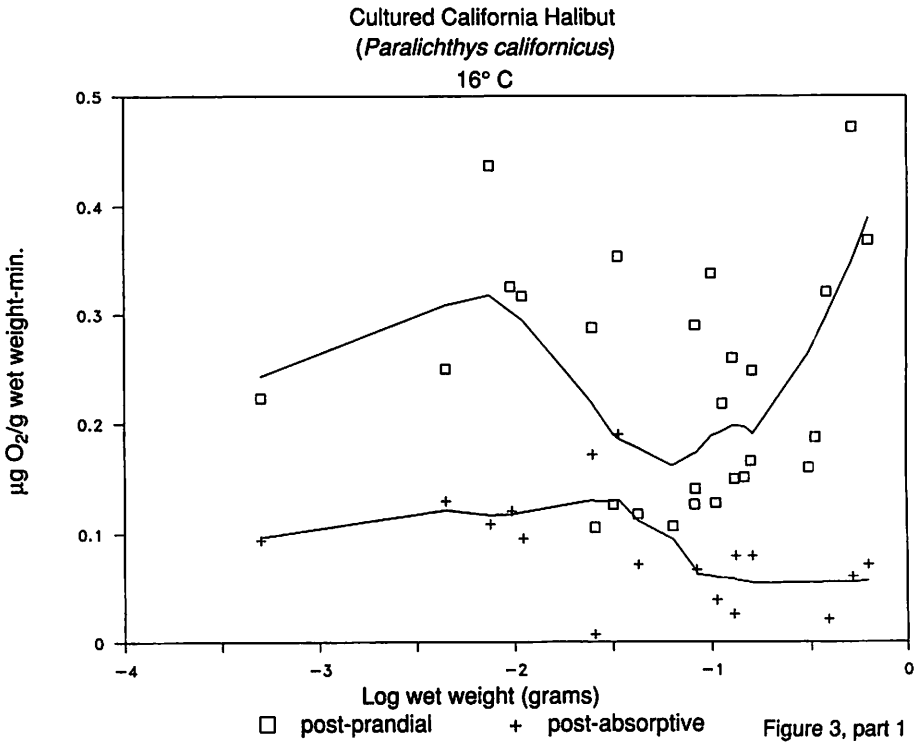


Figure 3, part 1

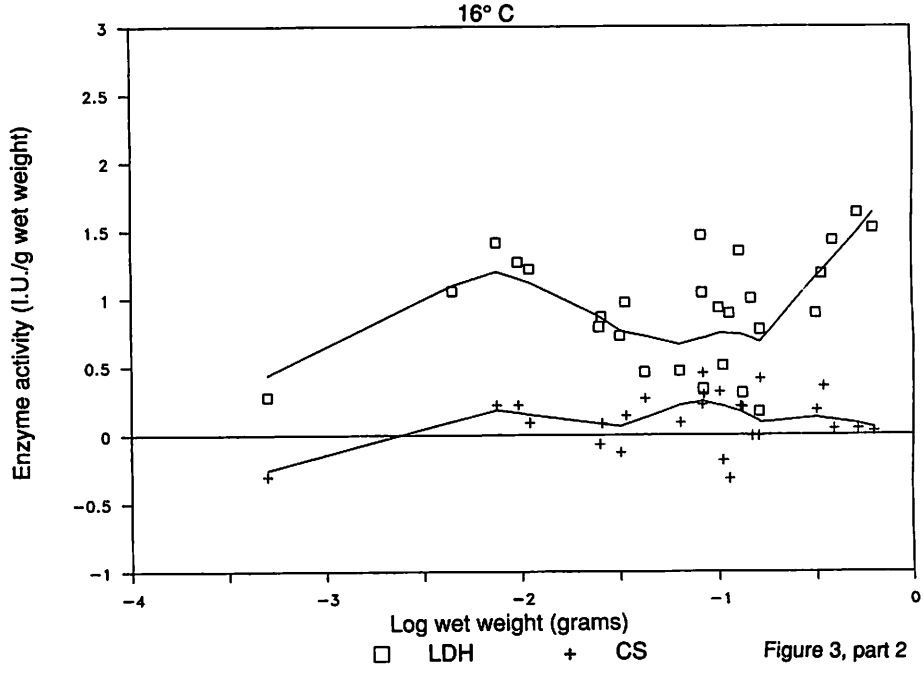


Figure 3, part 2

Figure 3. Measured metabolic rates as a function of wet weight (upper graph), and white muscle LDH and CS activity as a function of wet weight (lower graph). Metabolic scope as measured by a difference between postabsorptive and postprandial oxygen consumption as a function of the difference of the weight specific activities of CS and LDH in the white trunk musculature of the California halibut (2 graphs on next page). Notice that the slope of the relationship between metabolic scope and enzyme differences increases with increasing temperature.

metabolism (i.e., specific dynamic action). The respiration rate observed 24 hours later, after the completion of nutrient absorption and utilization in new growth, served as an estimate of resting metabolic rate. Figure 3 plots the difference between maximal and resting oxygen consumption rates vs. the difference between LDH and CS activities in white muscle. A statistically significant trend was found, indicating the validity of this index of metabolic scope. The effect of temperature on the postprandial metabolic rate was much greater than that measured in the postabsorptive state. This is evidenced by an increase in the slope of the relationship between the metabolic scope (as we have indexed it) and the difference in enzyme activities (i.e. LDH-CS activity) with temperature.

From the preliminary analysis of this experiment in progress, we have been able to establish the following: (1) There is a marked reduction in possible growth rate (at a given temperature) after the larval to juvenile transformation in California halibut (Figure 1). This was our prediction from the measurement of biochemical indices in the white trunk muscles of the fish made several years ago (as part of our previous Sea Grant project R/F-102). (2) The reduction in growth rate is a direct consequence of a reduction in the possible metabolic rates at this stage in the life history of the halibut (Figure 3). This was again predicted by our interpretation of our enzymatic assays of these fish. (3) Our biochemical indices for following recent growth and metabolic rate faithfully track actual changes in these rates in the halibut, despite the changes early in the juvenile phase of life. (4) When we complete our analysis of daily ration (from measured consumption rates and stomach volumes), we will be able to construct energy budgets for the halibut reared from 16 to 27°C. (5) This will enable our analysis of the biochemical indices of the several hundred field fish to be assigned quantitative values for growth and metabolic rate. Our growth-rate predictions should be corroborated independently by the analysis of otoliths from the same fish by Kramer and Hunter of the NOAA Fisheries Center, La Jolla.

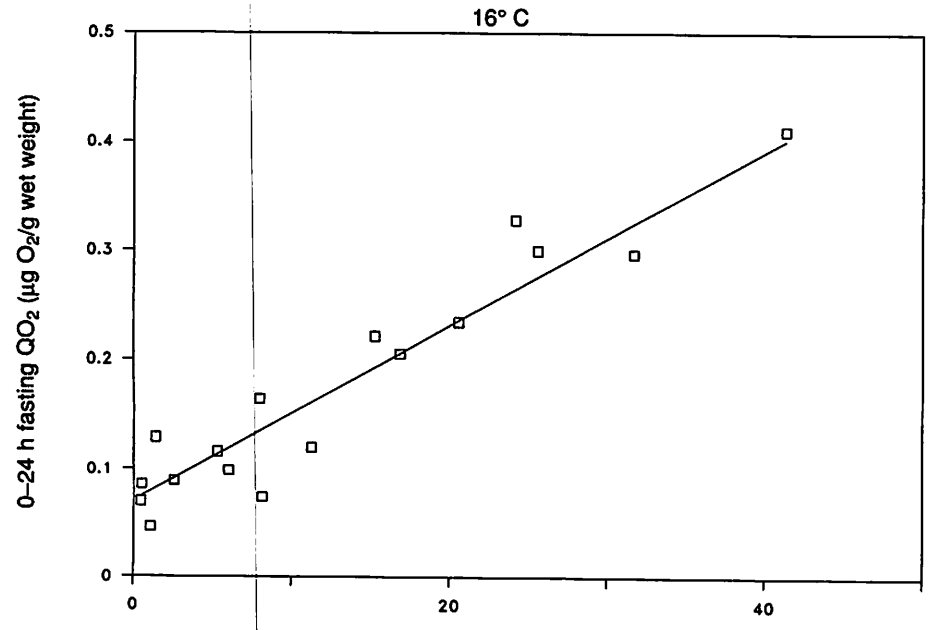


Figure 3, part 3

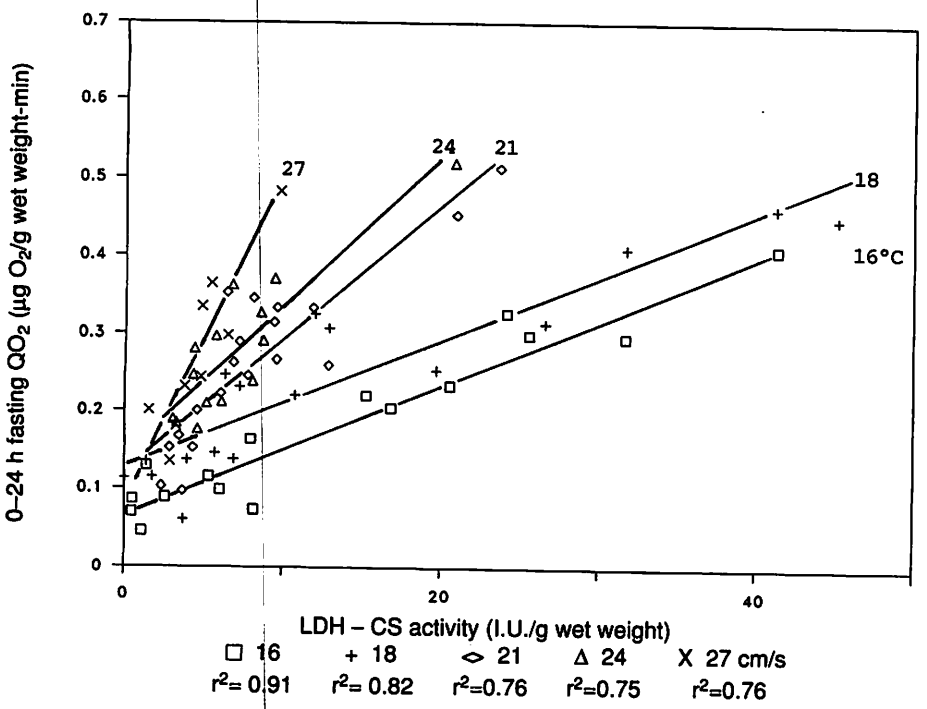


Figure 3, continued.

Figure 3, part 4

Cooperating Organizations

Occidental College
Southern California Edison Research and Development Laboratory
Southwest Fisheries Center, National Marine Fisheries Service, La Jolla

Reference

Preide, I. G. 1985. Metabolism. In *Fish energetics: New Perspectives.* P. Tytler and P, Calow, eds. Johns Hopkins University Press, Baltimore. Chap. 3.

Lectures

Kaupp, S. E. Ontogenetic change in the optimal temperature for growth in the California halibut. Paper presented at the California Cooperative Oceanic Fisheries Investigation meeting, November 1988.

Kaupp, S. E., and G. N. Somero. Biochemical indices of metabolic and growth rates in fishes. Presented at the American Society of Zoologists meeting, San Francisco, December 1988.

Kaupp, S. E. and G. N. Somero. The influence of temperature on growth and metabolic rates in larval and early juvenile California halibut. Presented at the California Halibut Symposium, convened by California Department of Fish and Game, San Pedro, May 1989.

New Marine Products

Insect Control Agents From Marine Organisms

University of California, Berkeley
R/MP-35
Project Initiated: October 1, 1985
Project Completed: September 30, 1987

Isao Kubo

Although nonchemical pest control shows promise, leading ecologists believe both chemical and non-chemical strategies are essential to food and fiber production. (Kriegen, 1982; McKelvey, 1972; Council on Environmental Quality, 1972). Many crops cannot be grown economically without the use of pesticides. Several conventional second-generation pesticides are proving less effective because of pesticide resistance evolving among arthropods, plant pathogens, and even rodents. Other popular pesticides accumulate in the food chain and in both ground and surface waters. Hence, there is a constant need to replace obsolete pesticides whose usefulness have expired because of evolved pest resistance, unsuitable toxicity, or an extended lifetime in the environment. Compounds isolated during this project will be used to supply agricultural chemical companies currently working with us with biologically active compounds not available from other sources. These new compounds, along with their potential new modes of action, can be used as synthetic leads for future pesticides.

The overall objective of this 2-year project (1985–1987) is to perform the bioassay-directed isolation of chemical compounds responsible for the observed pesticidal properties of selected marine algae. This is to be followed by characterization of their structure and a more thorough testing of their insecticidal properties against four major crop pests that our commercial collaborators have suggested. It is hoped these new compounds, along with their potential new modes of action, can be used as synthetic leads for future pesticides.

The objectives for 1985–86 were to complete the structural work begun on six algal compounds already purified and to test more thoroughly their range of insecticidal properties. We also planned to continue work on algal species (six Phaeophyta and ten Rhodophyta) already collected that had been shown to be active in our bioassays. Re-collection was to be performed as algal supplies were needed. In the last 12 months, extracts from another 10 different marine algae were tested against pink bollworm (*Pectinophora gossypiella*). The preliminary test indicated extracts from two species of algae *Callophyllis* sp. and *Botryocladia* sp. exhibit growth inhibitory activity against pink bollworm. Further purification of the active moieties from these extracts is now in progress.

The results from our previous studies show that there is still much to learn about the chemistry of marine algae and that our approach is capable of discerning different types of information than has been obtained in the past. We isolated the diterpene crinitol (*I*) in large amounts from the alga *Cystoseira crinita* by following an insect-feeding bioassay. We found *I* was active against pink bollworm (ED_{50} = 500 ppm; the ED_{50} is defined as the amount of sample required to cause 50% growth inhibition), which is an important crop pest in the United States. Although *I* had been isolated before (Fattorusso et al., 1976), its insecticidal activity was unknown. We also found it gave 100% growth inhibition of *Escherichia coli* at only 50 ppm. When compared with most commercial antibiotics, this would not be the level of activity of a strong bactericide. However, few antibiotics are active against gram-negative bacteria such as these, and we have been told that this activity is quite good. We have recently published this work (Kubo et al., 1985).

One of our commercial collaborators, Rohm & Haas, has asked that we supply them with enough crinitol to further test its spectrum of activity. They suggested that its lack of chlorine or bromine may make it more suitable as a model for terrestrial pesticides than other marine natural products that are halogenated. (The terrestrial environment has a history of problems dealing with halogenated compounds.) We have been contacted by representatives of a pharmaceutical firm about crinitol in reference to its antibiotic effect against gram-negative bacteria. They wish to investigate its use with contact lenses because it is common for lenses to become contaminated with gram-negative bacteria from the human gut. We have re-collected *C. crinita* so we can supply the large amounts needed for biological testing by these commercial interests. We have also collected closely related species, such as *Sargassum muticum*, in the chance they may evoke similar activities.

We identified the monoterpene *II* from a collection of *Plocamium cartilagineum* from Arcata, California. During the course of its isolation, its structure was reported from a collection of *P. cartilagineum* Santa Cruz, California (Crews et al., 1984). We found *II* was active in our artificial diet feeding assay, with an ED_{50} of 45 ppm against *P. gossypiella*. Similar halogenated monoterpenes were also present. Others have assumed from the similarities in structures that these other compounds would also be active as insecticides. We were able to show that these halogenated monoterpenes were not responsible for the observed activity of the crude

extract, and thus they were not pursued.

We isolated the bromophenol *III* from an extract of *Odonthalia flocosa*. The crude extract was relatively inactive in our insect bioassays. However, it is believed this alga was used by native American Indians to help grow crops. Rather than discard this extract, we performed a plant-growth bioassay. Eventually compound *III* was isolated and was shown to be responsible for a striking growth-stimulation effect. Our preliminary tests show that this compound can stimulate the growth of lettuce seedlings by 300%. Previously compound *III* had been discovered from *Rhodomela larix* (Katsui et al., 1967) and other species including *O. flocosa* (Fenical, 1975). However, no such activities have been reported. The mode of action of this compound is now under study in our laboratory.

Compound *I* has already resulted in one publication, and a paper describing the work on compound *III* has been submitted for publication. A paper about all three compounds was presented at the European Phytochemical Society in Switzerland in September 1986. This work was also presented at a meeting at the headquarters of Safer Chemical Corp., Victoria, B.C., Canada, in October 1986 and during a lecture series throughout South America that was sponsored by the United Nations. A paper dealing with a new NMR spectroscopic technique was recently submitted for publication as a result of trainee support. In addition, the Sea Grant trainee connected with this project has completed a Ph.D. Degree in chemistry related to Natural Products Chemistry.

Cooperating Organizations

Du Pont de Nemours, Inc.
FMC Corporation
Native Plant Resource Institute
Rohm & Haas
Safer Chemical Corporation
Suntory Institute for Bio-Organic Research
Zoecon Corporation

References

Council on Environmental Quality. 1972. Integrated pest management. Stock No. 4111-0010. Government Printing Office, Washington, DC.

Crews, P., S. Naylor, F. J. Hanke, E. R. Houge, E. Kho, and R. Braslau. 1984. Halogen regiochemistry and substituent stereochemistry determination in marine monoterpenes by 13-C NMR. *J. Org. Chem.* 49:1371–1377.

Fattorusso, E., S. Magno, L. Mayol, C. Santacroce, D. Sica, V. Amico, G. Oriente, M. Piatelli, and C. Tringali. 1976. Oxocrinol and crinitol, novel linear terpenoids from the brown alga *Cystoseira crinita. Tetrahedron Lett.* 12:937–940.

Fenical, W. 1975. Halogenation in the Rhodophyta, A review. *J. Phycol.* 11:245–259.

Katsui, N., Y. Suzuki, S. Kitamura, and T. Irie. 1967. 5,6-dibromo-protocatechualdehyde and 2,3-dibromo-4,5-dihydroxybenzyl methyl ether: New dibromophenols from *Rhodomela larix. Tetrahedron* 23:1185–1188.

Kriegen, V. H. 1982. Chemistry confronts global food crisis. *Chem. Eng. News,* December 20, p. 18.

Kubo, I., T. Matsumoto, and N. Ichikawa. 1985. Absolute configuration of crinitol. *Chem. Lett.* pp. 249–252.

McKelvey, J. J., Jr. 1972, Pest control in health and agriculture: International challenges. In *Pest control: Strategies for the future. National Academy of Sciences, Washington, D.C. pp. 7–17.*

Publications

Hanke, F. J. 1986. Natural products as a source for biologically active compounds. Doctoral dissertation, Department of Chemistry, University of California, Santa Cruz.

Hanke, F. J., I. Kubo, and S. Naylor. 1986. Evaluation of resonance assignments by their selective elimination. Submitted.

Kubo, I., F. J. Hanke, and W. F. Wood. 1986. Lanosol induces seedling growth stimulation. Submitted.

Kubo, I., T. Matsumoto, and N. Ichikawa. 1985. Absolute configuration of crinitol. *Chem. Lett.* pp. 249–252.

Lectures

Kubo, I. Natural products chemistry. Invited lecture series for Chile, Argentina, Uruguay, Paraguay and Brazil. United Nations Educational, Scientific and Cultural Organization (UNESCO). 1986.

Kubo, I. Pesticidal compounds from natural sources. Presented at meeting of the European Phytochemical Society, Switzerland, September, 1986.

Kubo, I. Naturally occurring insecticides. Presented at Safer Chemical Corp., Victoria, B.C., Canada, October 1986.

Molecular Probes for Improving Marine Algal Polysaccharide Quality

University of California, Berkeley
R/MP-36
Project Initiated: October 1, 1985
Project Completed: September 30, 1987

Watson M. Laetsch

The goal of this project was to develop molecular markers for improving the quality of marine algal polysaccharides. Monoclonal antibodies and hybridization probes are the two types of molecular markers prepared, characterized, and applied for the gelling subunits of carrageenan from red algae and alginate from brown algae. Hybridization probes are labeled short gelling subunits that bind to and identify gelling subunits in algal cell walls (Vreeland et al., 1987). The molecular markers will enable improved selection of strains, mutants and protoplasts for algal aquaculture; better screening of raw material for phycocolloid processing; and rapid screening of product quality and suitability for industrial applications. A major advance in addition to the molecular markers is the development of an electrophoretic system that permits rapid analysis of the size distribution of gelling subunits. The chain length and content of gelling subunits are both important for gelling properties. The molecular markers and electrophoretic assay developed during this project are valuable new tools for biotechnological advance in phycocolloid production and quality.

Carrageenan

Monoclonal antibodies were prepared against kappa carrageenan (Vreeland et al., in press), the carrageenan subunit that forms firm gels. Iota carrageenan has a similar structure but forms weaker gels, and lambda carrageenan does not gel. Eighteen anticarrageenan antibodies were selected by screening with both quantitative enzyme immunoassay and cell wall-specific labeling by immunofluorescence (Figure 1). Studies on antibody specificity were carried out on six promising antibodies by using inhibition of enzyme immunoassay. Kappa, iota, and lambda carrageenans were used to inhibit antibody–kappa carrageenan binding as the reference system. Four patterns of antibody specificity were found (Table 1). One antibody was kappa-specific, one antibody was iota specific, several antibodies were specific for the gelling kappa and iota subunits rather than nongelling lambda carrageenan, and one antibody had lambda specificity. For all of these antibodies, the difference between kappa and iota subunits was enhanced by using subunit fragments as inhibitors. Inhibition by furcellaran (a partially desulfated relative of kappa carrageenan) was similar to the results for kappa carrageenan. The inhibiting and reference carrageenan samples were not pure because kappa and iota subunits occur together on hybrid molecules (Greer and Yaphe, 1984). This means that the

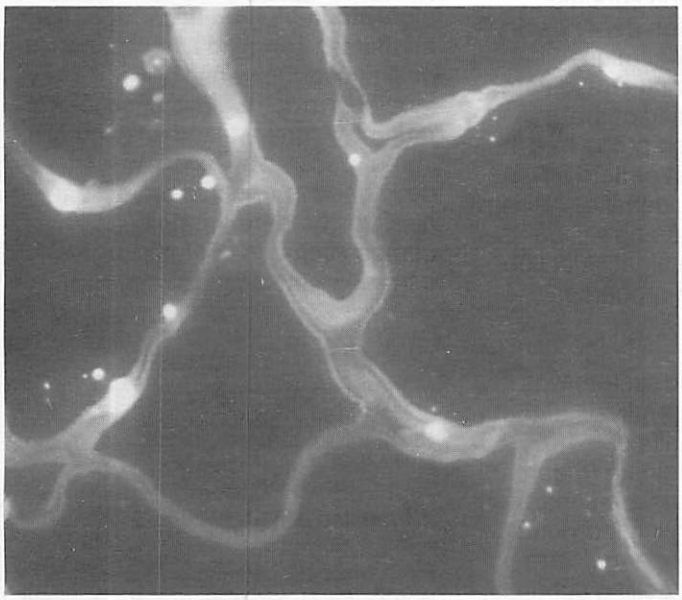


Figure 1. Monoclonal antibody 7E4-3F4 labeling of *Eucheuma alvarezii* cell walls. Indirect immunofluorescence on a methacrylate section.

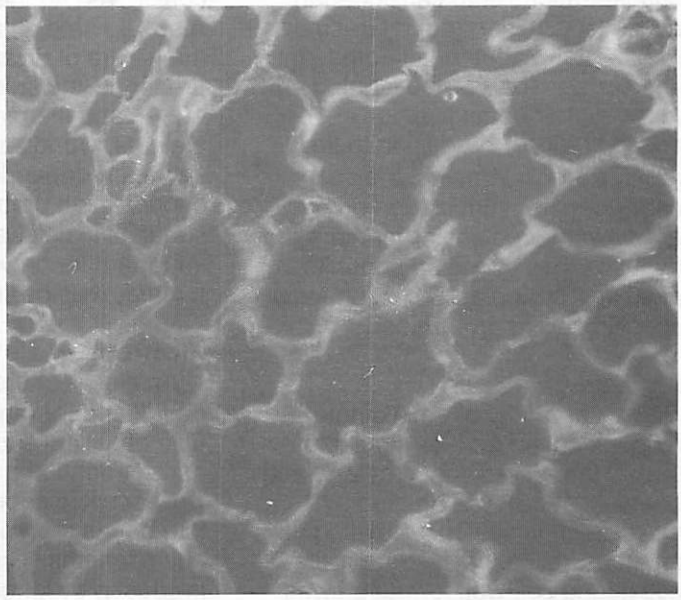


Figure 2. Labeling of *Eucheuma alvarezii* cell walls by the kappa carrageenan hybridization probe.

Table 1. Specificities of Anticarrageenan Monoclonal Antibodies

Antibody	Specificity Type	Kappa	Iota	Lambda
6AII	Kappa	1	50	60
10A5	Gel	1.1	1	78
7C2	Gel	1	1.7	233
5H12	Gel	1	3.6	600
3GI	Lambda	12	1.6	1
4D12	Iota	1.9	1	2.8

Note. Specificity was determined by an inhibition of enzyme immunoassay. Numbers represent the relative amounts of kappa, iota, and lambda carrageenan needed for 50% inhibition of antibody binding to kappa carrageenan.

specificities of the antibodies are likely to be even greater than shown in the table. These carrageenan monoclonal antibodies are excellent molecular markers for carrageenan source and product improvement.

A hybridization probe was prepared that specifically labels kappa carrageenan in cell walls (Zablackis et al., 1988). The probe consists of fluorescein-conjugated short-chain kappa carrageenan. It binds to kappa carrageenan in cell walls by the gelling mechanism in the appropriate ionic conditions (Figure 2). At 50 mM potassium chloride, only kappa carrageenan forms a gel; at higher potassium ion concentrations, iota carrageenan also gels (Smidsrød and Grasdalen, 1984). As expected, a fluorescent iota carrageenan probe labeled only at the higher potassium ion concentration. In addition to the monoclonal antibodies, the carrageenan hybridization probes are powerful molecular markers for the two types of gelling subunits in algal and protoplast selection for specific gelling properties.

Alginate

The specificity of antialginate monoclonal antibodies available from a recent project (Vreeland and Laetsch, 1986) was studied by localization inhibition. Labeling of antigens inside the *Fucus* zygote cell during cell-wall formation was inhibited by the G-block gelling subunits and M-block and mixed-block nongelling subunits. Three antibodies were identified as specific for gelling subunits and one antibody as specific for mixed blocks. This result established that alginate gelling subunits are produced intracellularly, although gelation is likely to occur in the cell wall (Vreeland and Laetsch, 1988, 1989). A hybridization probe for alginate gelling subunits was prepared (Vreeland and Laetsch, 1983) because the antialginate antibodies bind to the ends of alginate chains, which are seldom available in cell walls (Vreeland et al., 1987). The high degree of specificity of the hybridization probe for the gelling subunits of alginate was confirmed by lack of binding of fluorescein-conjugated nongelling subunits. Also, alginate gelation depends on the nature of divalent cations present (Smidsrød and Grasdalen, 1984), and the binding of the alginate hybridization probe had the same ion-dependent pattern. This hybridization probe identifies gelling subunits in cell walls and will be important for brown algal selection for improved gelling properties.

Electrophoresis

An electrophoretic system was developed for rapid analysis of the size of gelling subunits of carrageenan and alginate (Figure 3). Phycocolloid subunits are separated into bands representing individual chain lengths. Stronger gels are produced by longer gelling subunits, and for the first time the precise chain length and size distribution of gelling subunits can be easily displayed. In addition, multiple samples can be compared simultaneously, and information is gained on the purity of samples (minor bands are caused by contaminating subunits). This new gelling subunit electrophoretic assay is a breakthrough in the analysis of carbohydrate chain lengths in the gelling subunit size range and will be important in selection of phycocolloid strains, process monitoring, and product analysis. It is equally applicable to the pectate chains of higher plant pectins and other linear, charged carbohydrates.

Applications

The molecular markers and electrophoretic assay developed during this project were tested as new tools for improving phycocolloid raw material and products. The molecular markers for phycocolloid gelling subunits were applied to study alginate in the regenerating cell walls of *Fucus* protoplasts (Boyen et al., 1988) and carrageenan in *Eucheuma* cell walls (Zablackis et al., 1988). Antialginate monoclonal antibodies labeled *Fucus* zygote cell walls during cell-wall formation, and the labeling remained during germination. This established that labeling with molecular markers for gelling subunits can be used for selection of algal strains. The anticarrageenan antibodies can bind to carrageenan gelling subunits while gelled. They labeled the cell walls of *Eucheuma cottoni*. A quantitative enzyme immunoassay inhibition assay developed during this project can be used to compare the gelling composition and content of carrageenan samples.

Cooperating Organizations

Marine Colloids Division of FMC, Inc., Rockland, Maine
Kraft, Inc., Glenview, Illinois
NIH Mass Spectroscopy Facility, University of California, San Fransisco, Medical School

References

Boyen, C., B. Kloareg, and V. Vreeland. 1988. Comparison of protoplast wall regeneration and native wall deposition in *Fucus distichus* zygotes by cell wall labelling with monoclonal

antibodies. *Physiol. Veg.* 26:653–659.
Greer, C. W., and W. Yaphe. 1984. Hybrid (iota-nu-kappa) caarrageenan from *Eucheuma nudum* (Rhodophyta, Solieriaceae) identified using iota- and kappa-carrageenases and ^{13}C-nuclear magnetic resonance spectroscopy. *Bot. Mar.* 27:479–484.
Smidsrød, O., and H. Grasdalen. 1984. Polyelectrolytes from seaweeds. *Hydrobiologia* 116/117:19–28.
Vreeland, V., and W. M. Laetsch. 1983. Fluorescent alginate as a probe for carbohydrate-carbohydrate interactions in algal cell walls. *J. Cell Biol.* 97:416a.

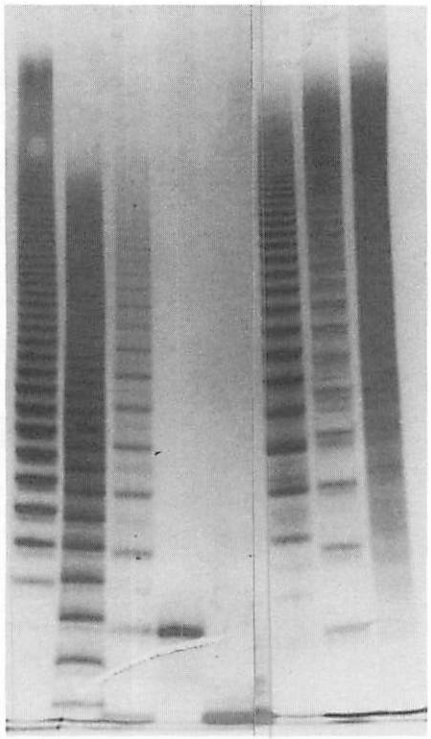


Figure 3. Polyacrylamide electrophoresis of carrageenan and alginate fragments. Each alginate band represents one oligomer length, and each subunit type has a distinctive band spacing. Kappa and iota carrageenan appear to behave similarly. Lanes 1–3: alginate G, M, and mixed blocks, respectively. Lanes 4–5: alginate mixed block calibrated standards, 10 and 8 sugars long, respectively. Lanes 5–8: kappa, iota, and lambda carrageenans, respectively, fragmented by acid hydrolysis. The faint banding by lambda is probably due to kappa contamination in this sample.

Vreeland, V., and W. M. Laetsch. 1986. *In situ* study of subunits of a plant cell wall carbohydrate during cell wall formation. *J. Cell Biol.* 103:243a.
Vreeland, V., and W. M. Laetsch. 1988. The role of alginate self-associating subunits in the assembly of *Fucus* embryo cell walls. In *Self-associating architecture.* J. Varner, ed. Allan R. Liss, New York. pp. 77–96.
Vreeland, V., E. Zablackis, B. Doboszewski, and W. M. Laetsch. 1987. Molecular markers for marine algal polysaccharides. *Hydrobiologia* 151/152:155–160.
Vreeland, V., E. Zablackis, and W. M. Laetsch. 1988. Monoclonal antibodies to carrageenan. In *Algal Biotechnology.* T. Stadler et al., eds. Elsevier, New York. pp. 431–439.
Zablackis, E., V. Vreeland, B. Doboszewski, and W. M. Laetsch. 1988. Localization of kappa carrageenan in cell walls of *Eucheuma alvarezii* var. Tambalang with *in situ* hybridization probes. In *Algal Biotechnology.* T. Stadler, et al., eds. Elsevier, New York. pp. 441–449.

Publications

Boyen, C., B. Kloareg, and V. Vreeland. 1988. Comparison of protoplast wall regeneration and native wall deposition in *Fucus distichus* zygotes by cell wall labelling with monoclonal antibodies. *Physiol. Veg.* 26:653–659.
Vreeland, V., and W. M. Laetsch. 1987. Algal cell wall studies with carbohydrate subunit markers. *J. Phycol.* 223 (Suppl.):12. (Abstract).
Vreeland, V., and W. M. Laetsch. 1989. Identification of associating carbohydrate sequences with labelled oligosaccharides. *Planta* 177:423–434.
Vreeland, V., E. Zablackis, B. Doboszewski, and W. M. Laetsch. 1987. Molecular markers for marine algal polysaccharides. *Hydrobiologia* 151/152:155–160.
Vreeland, V., E. Zablackis, and W. M. Laetsch. 1988. Monoclonal antibodies to carrageenan. In *Algal Biotechnology.* T. Stadler et al., eds. Elsevier, New York. pp. 431–439.
Zablackis, E., V. Vreeland, B. Doboszewski, and W. M. Laetsch. 1988. Localization of kappa carrageenan in cell walls of *Eucheuma alvarezii* var. Tambalang with *in situ* hybridization probes. In *Algal Biotechnology.* T. Stadler, et al., eds. Elsevier, New York. pp. 441–449.

Lectures

Vreeland, V. Molecular markers for plant carbohydrates. Invited lecture series, Department of Biological Sciences, University of California, Santa Barbara, April 28, 1986.
Vreeland, V. Molecular markers for marine algal polysaccharides. Invited minisymposium, 12th International Seaweed Symposium, Sao Paulo, Brazil, July 31, 1986.
Vreeland, V. Carrageenan molecular markers and electrophoresis. Invited lecture, Marine Colloids Division of FMC, Inc., Rockland, Maine, December 5, 1986.
Vreeland, V. Molecular markers for cell wall carbohydrates in plant cell wall development. Invited lecture series, Department of Molecular and Physiological Plant Botany, University of California, Berkeley, April 10, 1987.
Vreeland, V. The role of alginate self-associating subunits in the assembly of *Fucus* embryo cell walls. Invited symposium, Society for Developmental Biology, St. Paul, Minnesota. June 22, 1987.
Vreeland, V. Monoclonal antibodies to carrageenan and electrophoretic analysis. Invited lecture, Marine Colloids Division of FMC, Inc., Rockland, Maine, June 26, 1987.
Vreeland, V. Algal cell wall studies with carbohydrate subunit markers. Invited symposium, Phycological Society of America, Columbus, Ohio, August 12, 1987.
Vreeland, V. Monoclonal antibodies to carrageenan. Symposium, Recent Progress in Algal Biotechnology, Lille, France, September 17, 1987.
Vreeland, V. Identification of complex algal polysaccharides. Marine Science Institute, Department of Biological Sciences and Marine Biotechnology Program, University of California, Santa Barbara, May 16, 1988.
Vreeland, V. Analysis of phycocolloid structure and synthesis with molecular markers and electrophoresis. School of Botany, University of Melbourne, Australia, June 29, 1989.
Vreeland, V. Analysis of phycocolloid structure and synthesis with molecular markers and electrophoresis. Marine algal summer course, Hopkins Marine Station, Stanford University, Pacific Grove, July 12, 1989.
Vreeland, V. Monoclonal antibodies identify intracellular synthesis of carrageenan subunits. International Seaweed Symposium, University of British Columbia, Vancouver, Canada, August 15, 1989.

Vreeland, V. Electrophoretic analysis of alginate substructure and properties. International Seaweed Symposium, University of British Columbia, Vancouver, Canada, August 17, 1989.

Vreeland, V. Electrophoretic analysis of carrageenan substructure and interactions. International Seaweed Symposium, University of British Columbia, Vancouver, August 18, 1989.

Vreeland, V. Hybridization probes and electrophoretic analysis of polyuronates (pectins and alginates) in plant cell walls. Department of Pomology, University of California, Davis, August 30, 1989.

Zablackis, E. A kappa carrageenan hybridization probe. Symposium, Recent Progress in Algal Biotechnology, Lille, France, September 17, 1987.

Ocean Technology

Capsizing of Semi-Submersible Platforms

University of California, Santa Barbara
R/OT-12
Project Initiated: October 1, 1984
Project Completed: September 30, 1987

Jean-Louis Armand

Among the various types of mobile offshore drilling units (MODUs) currently used in the exploration and exploitation of offshore gas and oil fields and mineral deposits, semisubmersible rigs, until recently, had a good safety record. In particular, semisubmersibles were regarded as inherently safer than ships because of the their geometrical spread and moderate motions. And it is generally true that the capsizing of an intact semisubmersible rig is a highly unlikely occurrence under normal and even severe environmental conditions.

The situation, however, may change completely for damaged semisubmersibles in which structural, systems, or operational failure has significantly altered the initial stability conditions. At least two recent and dramatic accidents that have resulted in heavy losses of life, followed by public outcry to impose more stringent safety regulations for offshore drilling rigs, have shown that the seemingly large reserve of intact stability in semisubmersibles can be deceptive and also that existing regulations on damage stability are inadequate. A partial failure leading to modification of the stability characteristics must be recognized as a likely event, and every possible step should be taken to ensure that it does not result in the total loss of the rig.

Evaluation of stability characteristics of semisubmersibles is based on hydrostatic calculations only. However, the stability of a semisubmersible is essentially a *dynamic* problem, and every effort must be made to ensure that as much relevant dynamic information as is practicable is incorporated in studies of the behavior of semisubmersibles. This is, as is now known, particularly important in the case of a damaged semisubmersible.

The dynamic behavior in waves of semisubmersibles having a large permanent list after structural, systems, or operational failure has hardly been investigated. The few experimental results reported show the importance of nonlinearities in the equations of motions.

The main difficulty arises in the computation of the forces acting on the partially emerged pontoons. Impact forces, in particular, must be assessed because their influence may be considerable.

The problem of the vertical entry of a rigid horizontal cylinder into an incompressible inviscid fluid initially at rest has been considered (Armand and Cointe, 1986, 1987). Simplifying assumptions have been introduced, and the problem has been formulated. The method of matched asymptotic expansions has been used to solve the resulting boundary-value problem. A formula giving the impact force has been derived, representing a modification of the classical von Kármán formula. The results obtained have been compared with those of experimental investigations and numerical calculations. It has also been shown how the method can be extended to different geometries and nonvertical velocities to provide an estimate of the impact forces on the partially emerged pontoons of damaged semisubmersibles.

The next step was to investigate the impact of waves against elements of the structure. Wave impact on offshore structures has essentially been studied for small tubular members in the splash zone, or for spherical buoys. Its influence on the stability of damaged semisubmersible platforms in heavy weather conditions may be important. A simple model for the study of the impact of spilling breakers on a structure, based on a physical and mathematical model first described by Tulin and Cointe (1986), has been developed (Cointe, 1987a, 1987b). It shows how the problem reduces to that of determining the impact of a rigidly moving mass of water on a structure. The problem has been solved in two dimensions for small penetration by using the method of matched asymptotic expansions and extending the results of early work (Armand and Cointe, 1986, 1987). The theoretical results obtained were compared with experiments and emipirical formulae used in practice.

These results can now be applied to simulate the most general motion of a semisubmersible platform in waves. The platform is modeled as a rigid body with six degrees of freedom. The pontoons and the columns are decomposed into elements on which force calculations are performed, in the damaged as well as undamaged configuration, following a method similar to that developed by Paulling (1977). Nonlinear effects, such as those resulting from the impact of the pontoons on the free surface or the impact of breaking waves on the structure, can be introduced in a consistent manner.

The mathematical model must be validated and further refined by using available full-scale data as well as data obtained from model testing. To this effect, an elaborate model of a semisubmersible platform was built. It was designed as a 1:100 scale of the Ocean Ranger (1.2 m long, 0.75 m wide). These dimensions are compatible with the width (4.2 m) and depth (2.4 m) of the tow tank of the Ocean Engineering Laboratory at the University of California, Santa Barbara (UCSB), where experiments were conducted. The Ocean Ranger design was chosen

as representative of most current semisubmersibles and also because of the large amount of available data concerning this particular design. A precise scaling of the platform was made, and the weights were carefully distributed. Particular attention was given to the deck and the pontoon/ballast system.

A remotely controlled ballast system enables the alteration of the position of the center of gravity of the platform as well as of its trim and therefore allows the realistic simulation of damaged conditions. Another important consideration is the control of the natural roll, pitch, and heave frequencies, which are directly related to the metacentric height and should be in proper proportion to that of a full-scale rig. Mooring lines have also been included.

The model was constructed primarily from acrylic to provide a transparent structure, facilitating the assessment of the ballast. A remotely operated ballast system is a unique feature of the model. It enables tests simulating dynamic flooding in a realistic manner. Before its use in the newly constructed UCSB wave tank, the model was tested and tuned in the flume at Scripps Institution of Oceanography.

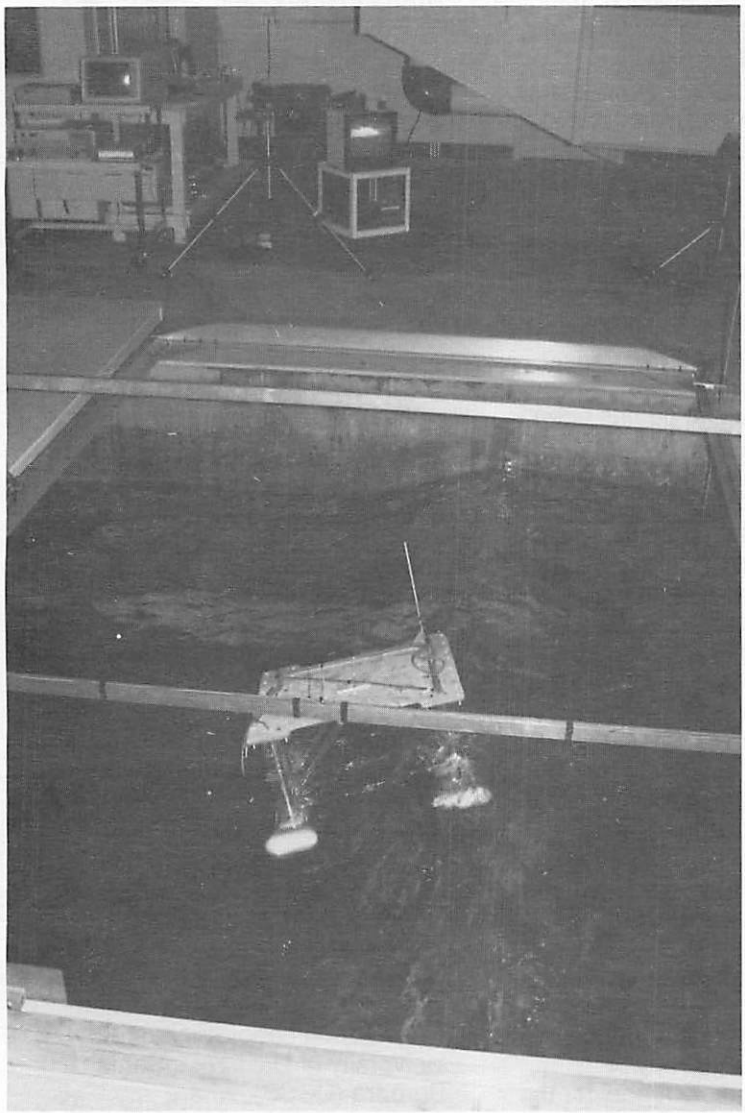


Figure 1. Experimental setup of Ocean Ranger model in listed configuration subject to large-amplitude planer wave.

Two methods were devised for accurate measurement of the motion of the platform. One records the accelerations; the other records the position of the model in time. The accelerators are triaxial and linked to a microcomputer through a data-acquisition system. The optical-motion measurement system uses two high-resolution video cameras, a VHS video recorder, and an image capture board mounted in a microcomputer. The system transforms the two-dimensional position of the six lights mounted on the model from the two cameras into the three-dimensional position in the fixed coordinates of the wave basin. Each frame, representing one time step, is then processed on a computor to yield the spatial configuration of the semisubmersible. The system is analogous to commercially available systems, but it runs at a significantly smaller cost and also produces a visual record of the tests. This method, adaptable to other experiments of rigid bodies in motion, is much more cost-effective than three-dimensional optical measuring techniques requiring the use of sensors.

The motion is first analyzed in two dimensions by placing the semisubmersible with pontoons perpendicular to a planar wave, in a damaged or undamaged configuration. The wave is created by a wedge, located at one end of the tank, which is 50 m long, and controlled by a hydraulic system. The length, period, and amplitude of the wave can be adjusted. A conical wave maker, designed by Tulin and Kolaini (1986), capable of generating three-dimensional waves is currently under construction; it will be used to analyze the response of the structure under the most general conditions.

The semisubmersible is moored at the bottom of the tank by nylon lines simulating the actual anchoring system. Special sensors were designed to measure the three components of the tension force exerted by the lines on the

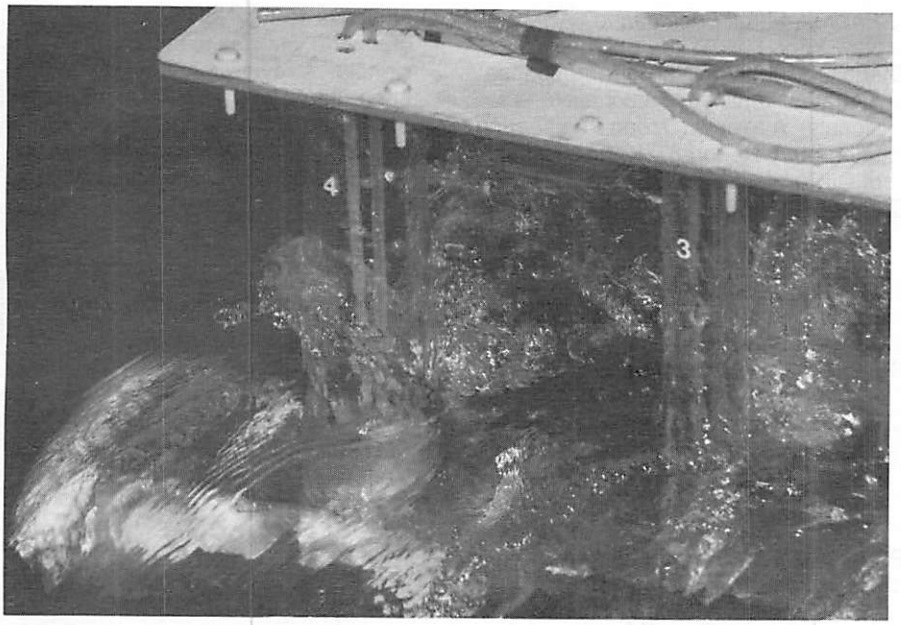


Figure 2. Breaking wave on pontoon of Ocean Ranger model.

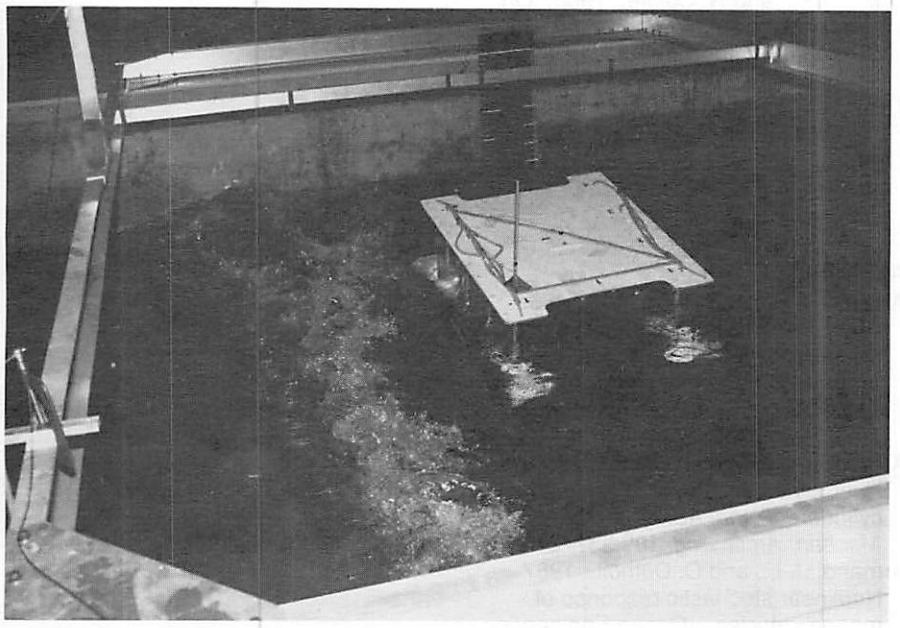


Figure 3. Breaking wave approaching Ocean Ranger model.

experimental model.

The set of data obtained was compared with the results of numerical calculations performed by using the code for large-amplitude motion of a listed semi-submersible developed within the framework of this research. Theoretical predictions were in good agreement with experimental results.

The actual capsizing of the semisubmersible is an extreme event that may be approached from the point of view of the general theory of nonlinear stochastic systems. No general theory exists at the moment to predict this response. An extensive review of available methods to predict the response of nonlinear systems to stochastic excitations compiled as a part of the present research (Duthoit, 1987) showed that the method of functional representation of the input-output relation in power series is one of the most widely used in seakeeping problems. It was also found that most techniques substitute one or several equivalent linear systems to the original nonlinear one.

These two observations were corroborated by theoretical and empirical evidence showing the superiority of the method of equivalent linearization to predict second-order statistics that contain the most important information to describe a random process. However, it was found that higher-order statistics cannot be predicted accurately within the framework of this technique whenever deviation from normal behavior becomes significant. A new technique was developed (Armand and Duthoit, 1987; Duthoit, 1987; Duthoit and Armand, 1987). It is based on the construction of a series of linear systems successively defined by linearizing the original nonlinear system and by matching the Volterra functional model response statistics of the desired order. The results obtained by using this technique are in good agreement with those obtained by the method of equivalent linearization when solving for second-order statistics.

Response probability distributions can be obtained once higher-order statistics are known. They provide the necessary information for the rational design of ocean structures as well as further insight into the statistical description of the various phenomena leading to capsizing.

Particular attention was devoted to the distribution of maximum entropy and its justification as a method of inference in problems with underdetermined response moments.

Applications to the roll motion of ships and the surge motion of a tension-leg platform were made and exemplify as well as assess the accuracy and the versatility of the overall method. Predicted response distributions of maxima were found to compare very well with digital simulation estimates.

In the course of this project, various complex phenomena

involved in the capsizing of a semisubmersible were modeled. An experimental, original setup provided some insight into the actual process of capsizing. A new analytical technique to predict the nonlinear stochastic response of marine vehicles was successfully developed and tested.

ARCO Exploration and Technology Company has expressed deep and continuing interest in the results of this research, which have been made available to international organizations such as the International Ship and Offshore Structures Conference and the International Towing Tank Conference. Extensive technical cooperation has also taken place with a variety of universities and organizations here as well as abroad.

Cooperating Organizations

ARCO Oil and Gas Drilling Company
Department of Naval Architecture and Offshore Engineering, University of California, Berkeley
Department of Naval Architecture and Ocean Engineering, Shanghai Jiao Tong University
Massachusetts Institute of Technology
University of Michigan

References

Armand, J.-L., and R. Cointe. 1986. Hydrodynamic impact analysis of a cylinder. In *Proceedings Fifth International Symposium on Offshore Mechanics and Arctic Engineering* 1:250–256. ASME, New York.

Armand, J.-L., and R. Cointe. 1987. Hydrodynamic impact analysis of a cylinder. *Trans. ASME J. Offshore Mechan. Arctic Eng.* 109:237–243.

Armand, J.-L., and C. Duthoit. 1987. Nonlinear stochastic response of marine vehicles. (Ocean Engineering Laboratory Report 87-17). University of California, Santa Barbara.

Cointe, R. 1987a. A Theory of breakers and breaking waves. Doctoral dissertation, Department of Mechanical and Environmental Engineering, University of California, Santa Barbara.

Cointe, R. 1987b. Two-dimensional fluid solid impact. In *Proceedings Sixth International Symposium on Offshore Mechanics and Environmental Engineering.* ASME, New York.

Duthoit, C. 1987a. Nonlinear response of marine vehicles to stochastic signals: A review (Ocean Engineering Laboratory Report 87-12). University of California, Santa Barbara.

Duthoit, C. 1987b. Nonlinear stochastic response of marine vehicles. Doctoral dissertation, Department of Mechanical and Environmental Engineering, University of California, Santa Barbara.

Duthoit, C., and J.-L. Armand. 1987. Nonlinear dynamic behavior of moored platforms driven by stochastic seas. In *Sixth International Symposium on Offshore Mechanics and Arctic Engineering.* ASME, New York.

Paulling, J. R. 1977. Time domain simulation of semisubmersible platform motion with application to the tension leg platform. In *Proceedings STAR Symposium.* SNAME, New York.

Tulin, M. P., and R. Cointe. 1986. On spilling breakers. In *Proceedings Sixteenth Symposium on Naval Hydrodynamics.* Berkeley, California.

Tulin, M. P., and A. R. Kolaini. 1986. Steep-short crested wave produced by a simple three-dimensional wave maker. In *Proceedings ATTC Conference.* Washington, D.C.

Publications

Armand, J.-L., and R. Cointe. 1986. Hydrodynamic impact analysis of a cylinder. In *Proceedings Fifth International Symposium on Offshore Mechanics and Arctic Engineering* 1:250–256. ASME, New York.

Armand, J.-L., and R. Cointe. 1987. Hydrodynamic impact analysis of a cylinder. *Trans. ASME J. Offshore Mechan. Arctic Eng.* 109:237–243.

Armand, J.-L., and C. Duthoit. 1987. Nonlinear stochastic response of marine vehicles. (Ocean Engineering Laboratory Report 87-17). University of California, Santa Barbara.

Cointe, R. 1987a. A Theory of breakers and breaking waves. Doctoral dissertation, Department of Mechanical and Environmental Engineering, University of California, Santa Barbara.

Cointe, R. 1987b. Two-dimensional fluid solid impact. In *Proceedings Sixth International Symposium on Offshore Mechanics and Environmental Engineering.* ASME, New York.

Duthoit, C. 1987. Nonlinear response of marine vehicles to stochastic signals: A review (Ocean Engineering Laboratory Report 87-12). University of California, Santa Barbara.

Duthoit, C. 1987. Nonlinear stochastic response of marine vehicles. Doctoral dissertation, Department of Mechanical and Environmental Engineering, University of California, Santa Barbara.

Duthoit, C., and J.-L. Armand. 1987. Nonlinear dynamic behavior of moored platforms driven by stochastic seas. In *Sixth International Symposium on Offshore Mechanics and Arctic Engineering.* ASME, New York.

Time Domain Analysis of Large Motions of Offshore Platforms

University of California, Berkeley
R/OT-14
Project Initiated: October 1, 1985
Project Completed: March 31, 1988

William C. Webster and J. Randolph Paulling

The major objectives of this project were to

(1) develop an understanding of some of the components of the hydrodynamic forces acting on a ship or ocean platform undergoing large-amplitude motions in high, steep waves,

(2) develop computer software that utilizes this knowledge in a numerical simulation of the large-amplitude motion of the floating body,

(3) ultimately to understand fully the mechanism of ship or platform capsize and, through this understanding, design guidelines for safer floating structures.

A computer simulation of large-amplitude motion of a ship moving in steep waves has been developed. During the course of development of this simulation, the important components of hydrodynamic force have been identified and the relative importance of each has been categorized. The simulation has been tested by comparison with the results of several field and laboratory experiments.

An investigation of the effects of hydrodynamic memory of the forces acting on a capsizing platform was conducted. This research included the development of a theoretical framework to incorporate these forces in a rational way into a capsizing simulation and the development of a computer program to perform the simulation.

The information generated by this work is being communicated to regulatory agencies such as the American Bureau of Shipping and the U.S. Coast Guard where it presumably will be used in the development of criteria for safety of floating vessels and platforms.

Publications

de Kat, J. O. 1988. Large amplitude ship motions and capsizing in severe sea conditions. Doctoral dissertation, Naval Architecture, University of California, Berkeley.

Hodges, S. B. 1988. Large amplitude roll motions of ships using multiple time scales. Doctoral dissertation, Naval Architecture, University of California, Berkeley.

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

University of California, Berkeley
R/OE-1
Project Initiated: October 1, 1986
Project Completed: September 30, 1987

Ben C. Gerwick and Weston T. Hester

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

Sea structures are subjected to high-cycle (more than 10^8 cycles), low-magnitude (up to 65% of ultimate strength) wave action over their entire life span. In addition, these structures are subjected occasionally to high-magnitude loads such as breaking storm waves and the impact of floating ice and other objects. For a typical concrete sea structure, high-cycle fatigue has not been considered a significant problem. However, low-cycle, high-magnitude fatigue is now recognized as a source of degradation, especially when there are numerous cycles into the tensile cracking range. Previous research done in Europe on fatigue behavior showed that a few high-magnitude cycles initiated cracks that were then reopened by the low-magnitude cycles, leading to accelerated fatigue failure, especially in the corrosive marine environment. It was also found that the submerged concrete was considerably more vulnerable to fatigue failure than the same concrete in air.

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

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

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

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

Goals

The goals of the project were to assimilate the state-of-the-art knowledge from different fields dealing with fatigue of engineering materials and arrive at the possible explanations and solutions to the aforementioned problems. A testing program on full-size beams and accompanying standard specimens was undertaken to evaluate the fatigue behavior of high-strength concrete under marine conditions.

Test Methodology

The fatigue tests were done under low-cycle, high-magnitude loading of concrete specimens with and without simulated marine environment. In selecting this method, we assumed that the low-magnitude fatigue is not a problem under these conditions, and we concentrated on the high-magnitude loads. Seawater (San Francisco Bay) was used when the testing called for immersion in water. Companion standard specimens were tested for all the mechanical properties of concrete, including compressive strength, splitting tensile strength, modulus of elasticity, modulus of rupture, ultrasonic pulse velocity, and bond with the reinforcing steel.

The following were taken into consideration when the methodology was selected: (1) The purpose of this test was not to arrive at exact engineering formulas, but to compare the behavior under fatigue loading of various materials. As long as the testing method was consistent and the comparison was done under the same accelerated conditions, the results should be valid. (2) The size of specimens had to be limited because of cost, capacity of dynamic jack, and so forth. (3) In choosing the appropriate cycling frequency, two concerns were important. It should be close to the actual frequency of 0.06 to 0.2 Hz, but the testing time for each set should not exceed 2 weeks. A frequency of 1 Hz was chosen and used throughout the test. (4) The actual loading sequence on marine structures in the ocean is random. However, testing under these conditions would be technically more difficult, and moreover, it would be impossible to make comparison with dry specimens and existing fatigue data. Therefore, constant stress levels were maintained. (5) The maximum stress level corresponded to 80% of static yield strength of the beams.

Conclusions

The tests led to the following conclusions:

1. Fatigue failure is a result of formation and propagation of

internal microcracks in the concrete.

2. Failure of the bond between reinforcing steel and concrete is the main variable in fatigue life.

3. For fatigue considerations, lightweight aggregate concrete is as good as or better than standard-weight concrete of similar strength.

4. No significant difference between fatigue life in air or in water was observed for high-strength concretes tested for low-cycle, high-magnitude cyclic fatigue.

5. Compressive strength does not control fatigue life.

6. Addition of silica fume to lightweight concrete doubled its fatigue life. The effect was due to improved bond strength between concrete and steel.

7. Addition of silica fume to normal-weight concrete did not affect the concrete's fatigue life. No improvement in bond strength between concrete and steel was observed.

8. High-strength, lightweight aggregate concrete is highly suited for use under cyclic loading.

9. The increase in fatigue life was not related to compressive strength, modulus of elasticity, modulus of fracture, or splitting tensile strength.

10. The observed pumping of water through cracks in the concrete that was tested submerged did not seem to reduce the concrete's fatigue life.

Only a few specimens of each concrete type could be tested under the reduced budget. The implications of the results of those that were tested are so important to future ocean structures that additional confirmatory tests are warranted.

Publications

Mor, A. 1987. Fatigue behavior of high-strength concrete under marine conditions. Doctoral dissertation, Department of Civil Engineering, University of California, Berkeley.

Mor, A., W. T. Hester, and B. C. Gerwick. 1989. Fatigue of submerged concrete under low-cycle, high-magnitude loads. *J. Testing and Evaluation* 17:(3):157–166.

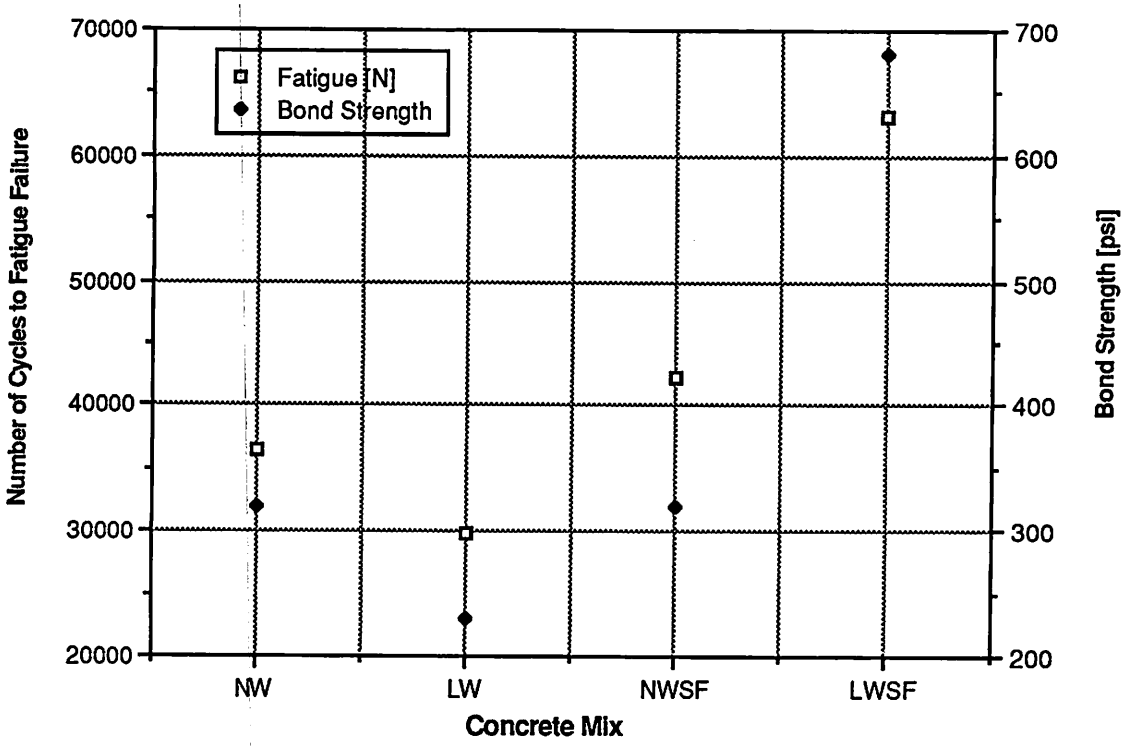


Figure 1. Effect of concrete mix and composition on fatigue life and on bond strength. NW = normal weight; LW = lightweight; NWSF = normal weight, silica fume; and LWSF = lightweight, silica fume.

Stability of Submarine Pipelines Against Breakout Failure

University of California, Berkeley
R/OE-2
Project Initiated: October 1, 1986
Project Completed: September 30, 1988

Mostafa A. Foda, Adrian W.-K. Law, and Jo Y.-H. Chang

During severe storm or hurricane conditions, wave-induced loading on submarine pipelines can be large enough to detach some of the placed pipes from the seafloor, causing them to move or float to great distances with the ocean currents. Numerous reports of this type of failure to submarine pipes are documented in the literature (e.g., Grace 1978). With the repeated storm-induced failures of marine pipes, the cost of maintaining their operational integrity can be quite high (e.g., Gerwick 1986). The needed improvement of the cost-effectiveness of the present design and maintenance practice for marine pipelines clearly can be established by gaining more insight into the various processes that constitute the failure mechanism.

In this work, we are studying the mechanical stability of the pipe-soil system under wave action. In particular, we have focused on the conditions that lead to pipeline detachment or "breakout" from the seafloor under hydrodynamic wave loading. We attempted to do that by simulating the breakout process in the laboratory and further identifying the critical wave-soil-pipe conditions for pipeline breakout. The experimental study was conducted for the configuration of a partially buried pipe in a sandy bed under shalllow-water wave forcing.

The experimental work was conducted in a wave flume 8 ft wide x 5 ft high x 180 ft long (2.4 x 1.5 x 54.9 m) at the Richmond Field Station of the University of California, Berkeley. The relatively large cross-sectional area of the flume allowed the building of a reasonable-sized sand basin inside the flume to model the seabed. A layout of the experimental setup is shown in Figure 1a. The flume is fitted at one end with a mechanical flap-type wave maker that generates monochromatic water waves, with a range of possible wave amplitudes and frequencies. At the other end of the flume, a 20° sloping beach with four layers of horse-hair mats is built to absorb the incoming wave energy. As shown in Figure 1, the test section is made of a rectangular sand basin 2 ft high x 8 ft wide x 4.5 ft long (0.6 x 2.4 x 1.4 m). Two 20° plywood ramps are fitted at both ends of the basin to provide smooth transitions for the water wave as it passes over the test section. The sand used in the experiments was a well-sorted, medium-sized, sand of D_{50} = 0.3 mm. The basin was filled with the sand, which was compacted manually in the dry, and then an 8-in. (20.3 cm) polyvinyl chloride pipe was placed across the flume, half-buried in the sand bed. The pipe was then connected to the various sensors of the experiment as needed, feeding into a data-acquisition system whose details are discussed in Foda et al. (1988). Then, the flume was filled with water to a total depth of 4 ft (1.2 m); this resulted in 2 ft (0.6 m) of water above the sand bed.

In all of the conducted experiments, the observed behavior was similar, consisting primarily of two distinct phases. First, there was the buildup phase, which covered more than 90% of the duration of the experiment. Then, there was the short phase of actual pipe breakout from the sand bed. During the buildup phase, there was hardly any discernible movement of the half-buried pipe. The only visible movement was that of some of the sand grains on the surface of the sand bed, which quickly resulted in the establishment of a regular ripple pattern on both sides of the pipe. However, in the near proximity of the pipe (about one pipe diameter distance on either side), no motion of sand grains was visible, and the sand surface there remained flat

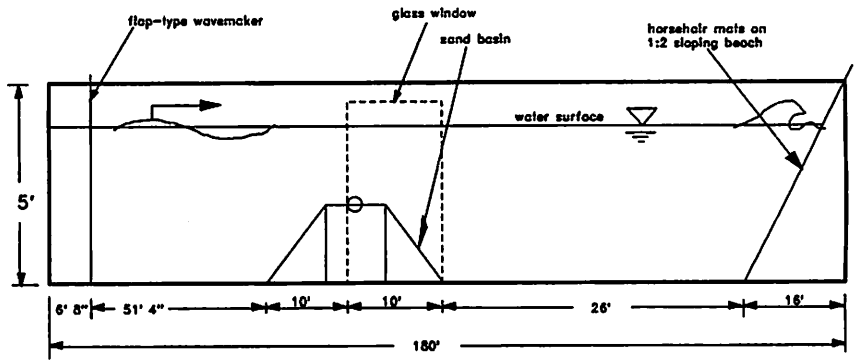


Figure 1a. A sketch of the flume dimensions.

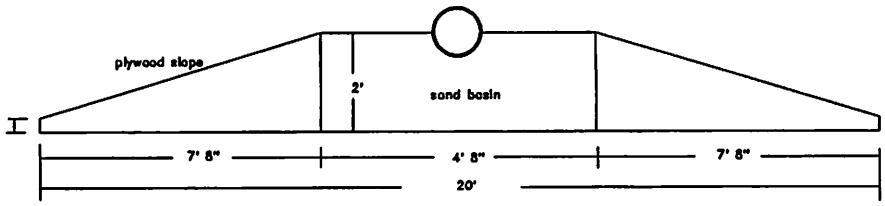


Figure 1b. The sand basin dimensions.

throughout this phase. In other words, in our setup, soil erosion did not play any role in the ensuing behavior of the pipe-sand system.

The duration of this buildup phase ranged from about 30 minutes to a little less than 5 minutes. During this phase, the pipe was clearly subjected to the hydrodynamic lift and drag caused by the passing of water waves above it. Flow separation took place in all the experiments. Separation was made visible by injecting liquid dye near the exposed surface of the pipe. The dye would quickly encounter and make visible one or more of the shedded vortices from the surface of the pipe. Such viscous separation would result in an asymmetric distribution of pressure around the pipe, and hence a net loading on it. Another cause for pressure asymmetry is the added-mass effect associated with the unsteadiness of the flow. Furthermore, there is the lift-force component associated with the Bernoulli's effect, where the increase in velocity at the summit of the pipe is accompanied by a decrease in pressure, (i.e., a lift). Figure 2 shows sample of the measured hydrodynamic forces on the pipe.

By examining the displacement record of the pipe during this initial buildup phase, we observed that the response of the pipe to these applied hydrodynamic loadings was in the form of a small oscillatory motion, with a slow net movement in the upward direction. Figure 3 shows the vertical displacement history of the pipe center during a typical run. In this figure, the amplitude of the pipe oscillation is about 0.008 in. (0.02 cm), and the average rate of rise of the pipe during this buildup phase is about 0.10 in./min (0.25 cm/min), which can hardly be detected by the naked eye. Superimposed on this gradual rise was an even slower rotational movement of the pipe around its longitudinal axis. This suggests that the pipe is actually rolling out of the sand bed, as opposed to being uniformly lifted off from the bed. With the wave coming from left to right, the observed net rotation was always counterclockwise. In other words, the combined rise and roll motion of the pipe did have an effective pivot point (point of zero net displacement), which was always at the wave-maker side from the pipe center. The rotational displacement was measured by placing markers at equal distances on the pipe wall and visually recording the differences between the readings at the two pipe-soil contact lines. This typically reached a maximal value, at pipe breakout, of about 10–15°.

Throughout this phase in which the pipe was slowly building up toward the ultimate breakout, the soil mass surrounding the pipe remained intact, with no sigh of any soil failure, such as soil sliding, erosion, or soil liquefaction. Finally, near the end of this buildup phase, the pulsating motion of the pipe became easier to detect by the naked eye, and this signaled the start of the short second phase of pipe breakout.

As seen in Figure 3, the slow, almost unnoticeable rise of the pipe during this long buildup phase was followed by the rather sudden and violent release of the pipe from the sand bed in the breakout phase. The release was so sudden that it was relatively easy to identify a specific time when it happened, and we called that the "breakout time." Figure 3 shows that approaching the breakout time, there was no significant increase in the amplitude of the oscillatory motion and that the breakout was essentially associated with the increase in the net rise component of the motion. This should quite reasonably exclude resonance as the possible mechanism for such a breakout. Instead, it is a quasistatic process through which the pipe gradually detaches itself from the sand bed, against the so-called mud-suction resistance force. The eventual breakout occurs because of the dissipation of this mud-suction force at breakout time.

Figure 4 shows the experimentally obtained data on the breakout force-time relation. The breakout force F_b is defined here as the net uplift force acting on the pipe, or the wave-averaged lift force minus the submerged weight of the pipe, and the breakout time t_b is determined from the recorded displacement histories (similar to Figure 3) when the slope of the curve reaches 90%

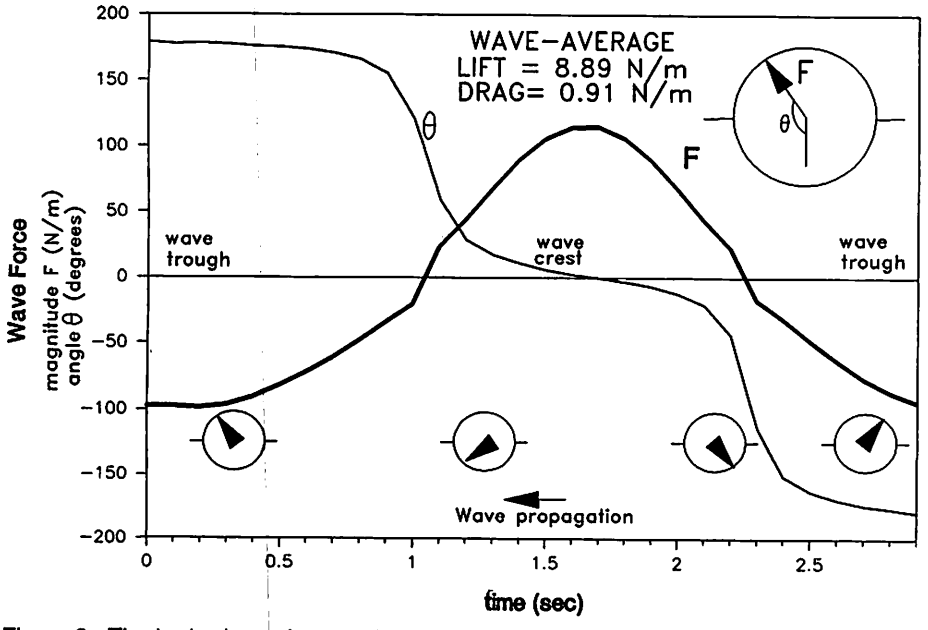


Figure 2. The hydrodynamic wave loading on the exposed half surface of the pipe, over a selected wave period (H = 0.2 m, T = 3 sec).

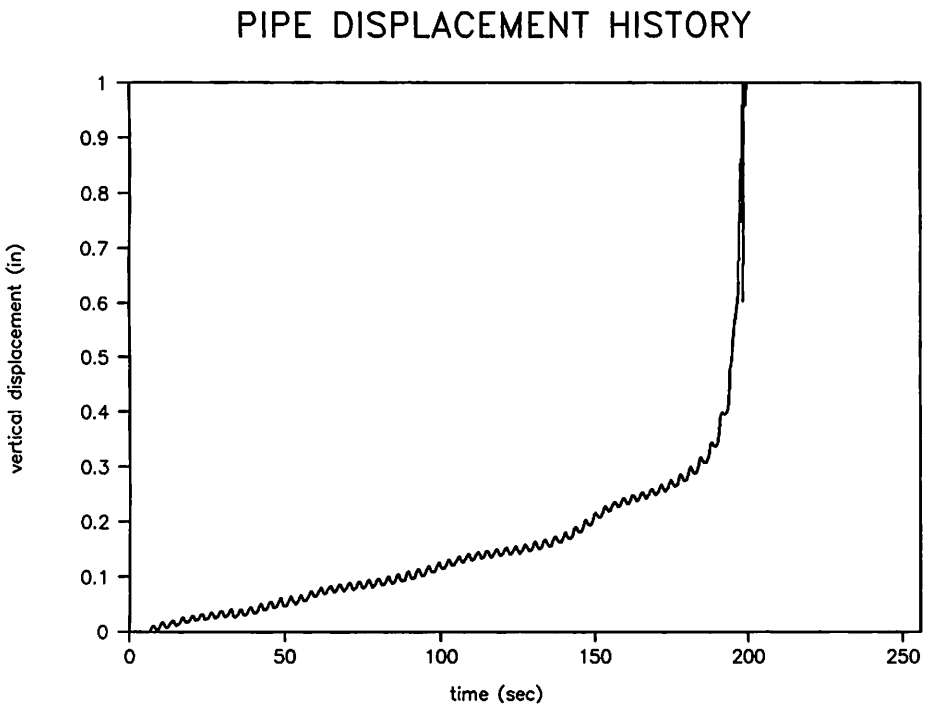


Figure 3. Vertical displacement history of the half-buried pipe under 6 in. (15.2 cm)–2.9 sec water wave.

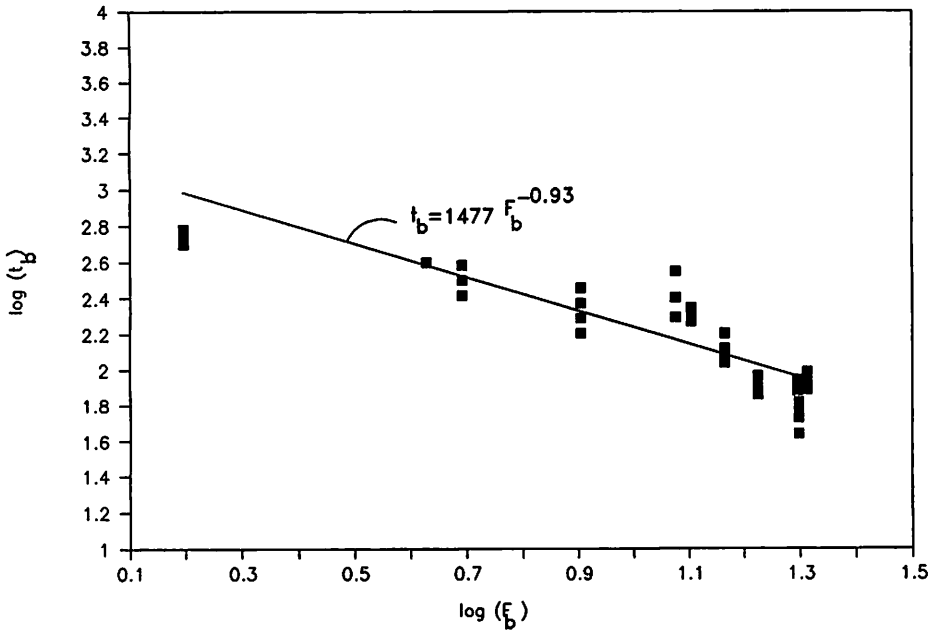


Figure 4. The experimentally obtained breakout force–time relation.

of vertical. The best-fit power law that runs through the experimental data was obtained by using a least-square routine, and is given in standard international units by

$$t_b = A\, F_b^{0.93};\ A = 1477 \qquad (1)$$

The exponent is very close to Mei et al. (1985) power law for breakout of flat-bottomed bodies from a porous rigid bed. Their law is given by

$$t_b = \tau(\mu L^{7/3} k^{-2/3})\, F_b^{-1} \qquad (2)$$

where μ is the water viscosity, k is the soil permeability, L is the contact width of the two-dimensional body, and τ is a nondimensional coefficient that ranges from 0.4 to 0.62, depending on the assumed slip boundary condition at the bed surface. By using conformal mapping, the aforementioned analytical relation was extended to our configuration of a half-buried pipe (Foda et al., 1989). The result was a modification in the value of τ from that for flat-bottomed bodies. For example, for a no-slip boundary condition at the bed surface, we get $\tau = 1.9$. The increase in the value of τ, and hence the breakout time t_b, is due to the nonuniform shape of the resulting gap between the pipe and the bed, with the smallest gap thickness being at the gap periphery. This will clearly reduce the lateral flux of ambient water through the gap periphery, as compared with the case of a flat-bottomed body, and hence delay the breakout time.

Now, recasting the constant coefficient A in Equation 1 to be of the form of the material constant of the law in Equation 2, where $\mu = 10^3$ kg/m-s, $k = 0.32 \times 10^{-10}$ m^2 (measured using a constant head permeometer), $L = \pi D/2$, and D = 0.2191 m, we find that the corresponding value of the free parameter τ is equal to $\tau_{exp} = 1.78$, which is very close to the theoretical value of $\tau = 1.9$.

References

Foda, M. A., A. W.-K. Law, and J. Y.-H. Chang. 1988. Wave-induced breakout of half-buried marine pipes. In *Proceedings, 21st International Conference on Coastal Engineering.* American Society of Civil Engineers, New York.

Foda, M. A., J. Y.-H. Chang and A. W.-K. Law. 1989. Submitted. Wave-induced breakout of marine pipes.

Gerwick, B. C., Jr. 1986. *Construction of Offshore Structures.* Wiley, New York.

Grace, R. A. 1978. *Marine Outfall Systems: Planning, Design and Construction.* Prentice-Hall, Englewood Cliffs, New Jersey.

Mei, C. C., R. Young, and K. Liu. 1985. Lifting of a large body from a porous bed. *J. Fluid Mech.* 152:203–215.

Publication

Foda, M. A., A. W.-K. Law, and J. Y.-H. Chang. 1988. Wave-induced

breakout of half-buried marine pipes. In *Proceedings, 21st International Conference on Coastal Engineering.* American Society of Civil Engineers, New York.

Numerical Bathymetry in Shallow Water

University of California, San Diego
R/OE-3
Project Initiated: October 1, 1985
Project Completed: September 30, 1988

Richard Seymour

The main objective of this project has been to evaluate with computer simulations a number of bathymetric sonar designs in order to select one best suited for very shallow-water (harbor) environments. For a bathymetric system to have a significant impact on the economics of harbor operations, certain performance requirements must be satisfied beyond what current operational systems can offer. Chief among them is the wide-swath requirement. A swath width of 7–10 times the water depth is thought necessary for efficient and economical harbor surveying. In addition, the potential of the design for implementation as a shipboard unit that can be mounted on a fast surface vessel is a critical factor.

Currently, two basic types of bathymetric technology have emerged. The first is based on the multibeam echo-sounder concept. Systems such as Sea Beam and Hydrochart II have been in operation for a number of years worldwide. Systems of this type produce reliable bathymetry over a swath typically 0.75–2 times the water depth. Thus, they are better suited for deep-water environments. The beams of the multibeam systems become progressively wider as they are steered away from the vertical and the aperture of the array is decreased. As a result, resolution suffers in the outer beams. This poses a fundamental limitation in the swath width attainable with multibeam systems. The second type of swath bathymetric system is the sidescan type; it measures depth by estimating the phase shift of the bottom arrival between two or more spatially separated receiving elements. Because the resolution is normally only a function of the transmitted pulse length, this concept can in principle achieve extremely wide coverage without loss of resolution in the outer reaches of the swath. On the other hand, a complete loss of useful data is incurred in a narrow strip in the near vertical. A small number of such systems (e.g., Sea MARC II, Sea MARC S, Bathyscan 300) are currently operational and have produced promising results with swath widths 3–4 times the depth. In contrast to multibeam systems that are shipboard, bathymetric sidescan systems exist only in a towed-fish configuration.

Guided by what is currently available in state-of-the-art bathymetric systems, we set out to develop a bathymetric concept that potentially could meet the stringent requirements imposed by the shallow-water scenario. Because of their complementary performance characteristics, we thought that a hybrid system, with a multibeam component in the near-vertical and a bathymetric sidescan component in the outer swath, would be a promising configuration.

Computer Simulations of Bathymetric Sidescan Sonar

Because the multibeam technology is a relatively mature one and is widely known to produce robust bathymetry, we decided to concentrate on evaluating the performance characteristics of the bathymetric sidescan component. The tool available to us for this purpose was the computer simulation program REVGEN (REVerberation GENerator). REVGEN produces synthetic reverberation according to the point-scattering model. In addition, it allows for realistic sonar system variables such as beam patterns and transmitted signals. The limitation of a flat bottom can be circumvented by using the option of inserting large numbers of arbitrarily located individual point-scatterers, thus creating additional bottom features.

During the first 12 months of the project, a large number of REVGEN simulations were performed involving a simplified bathymetric sidescan sonar system. Simplified transmitting and receiving beam patterns were used. The transmitting beam pattern was of the sidescan type, narrow in the along-track and wide in the cross-track direction. A total of four directional receiving elements were inserted at various vertical separations. The carrier frequency was set to 100 kHz and the pulse length to 1 ms. The sonar platform was placed 5 m above the bottom. The REVGEN output consisted of the acoustic return received during the first 0.125 s, sampled at 4096 Hz. This represented a swath width of approximately 180 m. Preliminary data analysis has shown that REVGEN can produce realistic simulations of the sidescan bathymetric concept. The behavior of the observed phase difference between receiver pairs as a function of range was consistent with the experimental geometry. Spatial aliasing resulted in phase discontinuities, as expected, for element separations larger than $\lambda/2$. Signal coherence was poor in the near-vertical, resulting in spurious bathymetry. This was not a concern because the near-vertical region was to be covered by the multibeam component of the system. Coherence quickly improved with increasing range, and the flat bathymetry could be readily recovered from the phase information.

The statistical behavior of the phase difference was consistent with the predictions of the point-scattering model. Occasionally, phase anomalies appeared, representing "outliers" in the

probability density function (pdf) of the phase difference, which is known to be a function of the spatial coherence between receiving elements. Combined ensemble and time averaging was effective in suppressing these phase anomalies. In addition, a phase bias effect became apparent. The bias resulted from phase aliasing when the phase mean approached maximal values ($-\pi$ *or* π). Simple averaging alone did not alleviate the bias problem. Bias correcting procedures have been suggested in the literature.

The initial simulation runs established that REVGEN is well suited for simulating the bathymetric sidescan concept. As a next step, we proceeded with three sets of simulation ensembles, each consisting of 30 pings and each designed to test the impact of environmental factors on the performance of the simple system configuration. In each case, a sound-speed profile constant with depth was assumed. Care was taken to include a sufficient density of bottom scatterers to create Gaussian reverberation at the receivers. A simple angular dependence function for the bottom-scattering strength coefficient was prescribed. No surface reflections and/or scattering were allowed, although we do recognize the potential impact of the surface contribution.

The first simulation served the function of a control experiment and represented the most benign scenario possible. Only bottom scatterers were inserted, and a flat bottom was used. After processing of the synthetic phase difference signal, and excluding *nadir*, the 5-m constant-depth profile was successfully recovered. Averaging was necessary to suppress the phase fluctuations and to produce a smooth profile.

The second simulation was designed to test the effect of sloping-bottom features on the quality of the bathymetric estimate produced by a bathymetric sidescan system. Here, we used the REVGEN option of inserting point scatterers at arbitrary locations to create sloping features on the flat bottom. Simple geometrical arguments indicate that the insonified bottom area will increase as a function of the bottom slope rather than being constant and only a function of the transmitted pulse length. As a result of the increasing vertical extent of the insonified area, reverberation theory predicts loss of coherence between the scatter received by two vertically separated receivers, which in turn results in increased variance in the phase estimate and potentially erroneous bathymetry. The simulations collaborated this prediction. For relatively gentle slopes, no degradation in bathymetric performance was observed. When the slope reached or exceeded a range-dependent "critical" value, considerable phase spreading was observed, and degraded bathymetry was produced. This type of effect has been encountered in real systems and has been termed the "glissando" effect. This simulation has served to quantify the significance of this effect.

The third simulation returned to the flat-bottom scenario but included a measure of volume reverberation. Because volume reverberation has directional information in it, it would be expected to have a biasing effect in the bathymetry. This indeed was the case. Volume reverberation led to bathymetric bias toward lower depths. Although volume reverberation has not been cited as the cause, this tendency of bathymetric sidescan sonar to slightly underestimate depth has also been reported elsewhere.

The fourth and final simulation of this initial set was identical with the third, with the exception that incoherent ambient noise was added. This had a deleterious effect on bathymetry in the far ranges where the bottom reverberation signal had decayed sufficiently as to fall below the ambient noise "floor." Clearly, ambient noise will be the ultimate limitation in the performance of bathymetric sidescan in the far ranges of the insonified swath.

Computer Simulations of a Hybrid Bathymetric Sonar

The first series of simulations was extremely helpful in identifying and assessing quantitatively the factors that limit the performance of a bathymetric sidescan system. On the basis of the existing data base, we proceeded to design a bathymetric sonar system that would address all known problems. The new design was a modified version of the original hybrid concept. The decision was made to maintain hybrid operation throughout the swath, rather than simply use a multibeam section in the near *nadir*. The reason for doing this was twofold: First, the original simulations showed that volume reverberation and ambient noise are harmful contaminants, especially when the bottom reverberation signal is weak. The presence of narrow beams throughout the swath will eliminate much of that interference, provided of course that the bottom return can be detected individually in each beam. Second, we thought that the availability of a full-fledged multibeam arrangement would result in another set of bathymetry, based on time-of-arrival information within each beam, at no extra cost. This was in the spirit of "testability by design" and would amount to a useful self-test. That is, the high-resolution sidescan bathymetry could be compared at all times with the low-resolution (in the outer beams) but perhaps more robust multibeam estimate. A third factor emerged when both the old (standard sidescan) design and the new (hybrid) system were subjected to multipath interference. Multipath is not a particularly serious problem in deeper water, but in the very shallow waters of harbors it is quite destructive and poses a very hard limit on the performance of the standard sidescan system. On the other hand, by concentrating the bottom return to narrow beams, the hybrid system should afford substantial protection against multipath returns.

Following this rationale, we designed and implemented in software a hybrid bathymetric sonar with the following characteristics: a transmitting array; frequency and pulse length identical to the old system; and two vertically separated arrays, $\lambda/2$ apart, each producing a total of 20 beams for each side of the swath. A one-sided (starboard) system was simulated, as port/starboard symmetry was expected. The beam forming done was conservative, in the sense that we readily allow for the increased beam width for the outer beams due to aperture limitations. The beam width increased from 3° (first 12 beams), to 6° (next six beams) to 12° (two outermost beams). Simplified beams with no sidelobes were used in this first implementation. A new generation of simulations was performed by using the new system. It was soon realized that the problem of detecting the bottom reverberation return individually within each beam necessitated sampling rates far exceeding the Nyquist rate. Thus, a sampling frequency of 40,960 Hz was used in place of the 4096-Hz rate used by the standard sidescan system. In fact, even higher rates would be desirable for better separation of the returns in the first few narrow beams. This immediately pointed to a drastically increased data rate. New software had to be developed to extract bathymetry separately from each beam and smoothly splice the estimates together from beam to beam. Once these problems were solved, the hybrid system was shown to produce reliable bathymetry for the simplest case of flat bottom with no interference present.

After a bottom-mapping algorithm was established, the hybrid system was subjected to volume reverberation and ambient noise interference. The results were largely as expected. The hybrid system performed similarly to the standard bathymetric design for relatively mild interference levels. However, as the volume reverberation level increased, a clear difference in performance emerged, with the hybrid system clearly outperforming its rival. Similarly, substantially better performance was achieved when ambient noise was the limiting interference. The bottom-tracking algorithm, however, had to be modified several times to reliably detect the bottom arrival as the interference levels increased. Clearly, the enhanced performance of the hybrid system was accompanied by increased demands in terms of the signal processing necessary. The problem of optimal detection of the bottom arrival in each beam is intimately coupled with the advantages offered by this configuration. By comparison, the standard sidescan design is strikingly simple in this regard.

A final series of simulations involved the effect of multipath interference. Allowing both bottom and surface reflections in the same shallow-water setting, we showed that the standard sidescan system was hopelessly limited to a swath width of twice the depth because of the onset of the bottom-surface-bottom return. The hybrid sonar performed much better under similar multipath conditions. Occasionally, multipath components did "leak" into the receiving beam, especially in the outer reaches of the swath where the beams got wider. Overall, however, this particular simulation provided the strongest support thus far for the choice of a hybrid system in a shallow-water environment.

Remarks

The REVGEN simulations of the hybrid systems were much more expensive computationally than the ones involving the standard sidescan system. A number of workstations, including a Sun 3/60, two Vaxstation 2000s, and a Microvax 3600, in addition to a Vax 750 computer, were dedicated to this project continuously for periods of several months. Duke University shouldered most of the computational expense at no added cost to the project. In addition, the Marine Physical Laboratory of the Scripps Institution of Oceanography contributed significant computing resources. As we approached the end of the simulation work, it became clear that a supercomputing facility would be necessary for further progress. This is now possible as REVGEN has recently been ported to run on the Cray-XMP machine of the San Diego Supercomputing Center. This conversion was funded by the Office of Naval Research, with this project providing some impetus. A speed-up factor of approximately 90 is the result. More realistic simulations (e.g., realistic beam patterns and bottom profiles) are desirable and now possible.

Throughout the simulation effort, we have ignored the effect of surface reverberation entering though the sidelobes. That should not be taken to imply that we do not consider surface reverberation to be a factor. In fact, it will quickly make the sidescan bathymetric concept useless if present in significant amounts. Our assumption is that everything possible will be done (as is in currently operational systems) to reduce the array response toward the surface through appropriate baffling. In terms of surface reverberation interference, our hybrid design is equally vulnerable as the simple sidescan system.

Effects that were too expensive to simulate include the effect of ray bending and vehicle motion. We hope to be able to include these in the future as the Cray version of REVGEN becomes fully operational.

Conclusions

This process of computer simulation has proved to be of great value in studying the performance of realistic bathymetric sonar configurations under various environmental conditions. It provides a bridge between general theoretical predictions offered by the point-scattering model of reverberation and reported performance characteristics of real-life sidescan bathymetric systems. Using REVGEN, a high-fidelity

sonar-system simulation program, we have tested a hybrid bathymetric design that promises significant advantages over current state-of-the-art systems in terms of swath coverage and robustness in the presence of interference, especially in shallow water. The added cost is in terms of increased complexity, both in system design and ensuing signal processing. Continuing the simulation effort in a supercomputing environment and joining forces with a group experienced in the hardware design and deployment aspects of the problem, we hope to set in motion a process that will result in an operational prototype of the hybrid concept.

Cooperating Organizations
U.S. Corps of Engineers

Marine Affairs

Forecasting Commercial Passenger Fishing Vessel Angler Participation

University of California, Davis
R-MA-27
Project Initiated: October 1, 1986
Project Completed: September 30, 1988

James E. Wilen and Warren Johnston

In the state of California, recreational anglers spend millions of dollars each year on gear, guiding services, party boat trips, motels, food away from home, and other foods and services associated with fishing activities. In addition, anglers play a role in, and contribute funds to, the management and protection of the state's freshwater and marine fisheries. Despite their obvious importance, however, little is known about recreational anglers' basic patterns of use; how their patterns of use are affected by the resource in question; and how, in turn, their patterns of use affect the resource. For example, has recreational angling been growing or declining over the past decade? How much of observed change in participation is due to population growth and demographic changes, and how much is due to changes in resource conditions? Do angler's successes affect participation rates from season to season, and if so, by how much? Are there important within-season patterns of participation? How do angler days translate into actual catch? An understanding of these types of questions would be beneficial to persons directly involved in activities servicing recreational anglers and to managers charged with maintaining a viable resource.

This research is investigating one important subgroup of recreational anglers–party boat anglers–in order to address some of the aforementioned questions. Party boat angling is particularly promising to study because of the high-quality data sources derived from logbooks and maintained on computer files by the California Department of Fish and Game. These data sources are being subjected to statistical modeling to determine factors that have affected participation rates between and within seasons over the past decade. In addition, we are examining how participation rates determine catch in order to understand the nature of resource/participation interaction. A clear understanding of these factors will enable user groups, service industries, and managers to better forecast trends in recreational angling.

The overall objective is to analyze data on California party boat angling to better understand factors responsible for trends in participation rates. Data have been obtained from log book files (with due attention to confidentiality requirements) and sorted to provide a time series from 1976 forward of ports and species-specific records of anglers' participation rates, effort levels, success, and so forth. These data are being subjected to standard statistical and econometric methods to separate out the effects of changes in factors between seasons (e.g., general abundance), within seasons (e.g., relative abundance timing, weather, holidays), and between regions and targeted species. The results will be a series of models that predict total participation levels in various ports, by targeted species, on a weekly or monthly basis within the season. In addition, we will analyze the corresponding relationships between actual participation rates, total effort levels, and catch rates.

We have obtained tapes covering 1976–1986 (except 1979, which we are in the process of obtaining) for the whole of California. Data have been sorted and files developed that aggregate (on a weekly basis for each of the ports) information on anglers' participation (e.g., number of anglers, angler hours, trips, vessel hours), catch by species, and success measures currently and in the recent past (e.g., catch per angler hour, catch per angler, catch per angler trip).

The modeling phase is still in progress. The data have been sorted to analyze sportfisheries for salmon, albacore, and bottomfish. Our efforts to date have focused on the salmon fishery in eight northern California ports (responsible for 95% of the total sportfishery catch). Two sets of forecasting equations have been estimated: one examining total angler catch (by week) as a function of effort and the second analyzing angler participation as a function of recent success rates in each port. The results of these preliminary analyses suggest the following: (1) The elasticity of total catch with respect to total angler trips is surprisingly large (greater than one). (2) A simple relationship between total weekly catch (by port) and total angler trips provides good predictive power of sportfishing catch. (3) Angler participation is responsive to last week's success, with elasticities up to + 0.5 for Chinook-dominated urban ports in San Francisco. (4) Anglers' response to success in Northern Coho–dominated ports is low or statistically insignificant. (5) Total angler participation in all ports is influenced by a "summer peak effect" that seems uniform across all ports.

The remaining tasks to be accomplished will follow the methods tested in the salmon fishery. We intend to complete similar analyses for the albacore fishery and for subsets of the groundfish fishery. Results will be disseminated in one or more journal articles in the marine economics literature.

Cooperating Organizations
California Department of Fish and Game

Lecture

Wilen, J. Angler participation response in the salmon sportfishery. Invited Lecture, Department of Fisheries and Oceans Seminar Services, Vancouver, British Columbia, January 1988.

The United States, Japan, and the Pacific Fisheries: Economic Relations, Diplomacy, and Ocean Law, 1945-85

University of California, Berkeley
R/MA-28
Project Initiated: October 1, 1986
Project Completed: April 30, 1988

Harry N. Scheiber

This project is an investigation of the history of relations between Japan and the United States since World War II. It embraces the complex interrelationships of changing marine science and marine resources management concepts, diplomatic objectives, and evolving international law. The central objective of the research is to enhance public and scholarly understanding of the lessons for policy that this historical record offers. By intensive examination of the experience in U.S. relations with Japan since World War II, this study will cast light on an economic and political relationship that has become one of the dominant factors in Pacific Basin affairs in our own day—and will remain so for the foreseeable future.

Objectives for the research included completion of research already commenced before October 1986 on material in the archives of the U.S. State Department and in papers of individual scientists and participants in diplomatic and Law of the Sea discussions. Survey, copying, and analysis of data relating to expansion of the Japanese distant-water tuna enterprise and whaling, and relating to scientific research in relation to commercial rivalries during the postwar era, have been undertaken. The materials exploited include newspapers and journals and publications on international trade and on Law of the Sea and international law generally.

Previous scholarship on Japanese-American diplomacy (e.g., Borden, 1984), despite an emphasis on Japanese economic recovery in the 1940s and expansion in the 1950s, typically gave little or no attention to fisheries policy issues. The current project has made a central focus of attention research on the complex interrelations of science, economic change, and diplomacy in regard to fisheries. Research involving the papers of individual scientists, agency material in the official National Archives collections of the Pacific Oceanic Fishery Investigations (POFI, the Hawaii-based tuna investigation of the U.S. Fish and Wildlife Service), and material gathered from interviews and writings in Japan, have illuminated the role of fisheries issues as a central issue in relations since War War II.

One of the main themes emerging, and on which additional intensive research is required, is the role of organized industry initiatives in the policy process surrounding the tuna rivalries of the United States and Japan: competition for development of new fishing areas in the Pacific and in scientific work of exploration and survey, rivalry in regard to markets, and complexities arising from the variety of Japanese tuna products aimed at the U.S. domestic market and the differential impact of these products on sectors of the U.S. marine industries. A large body of American Tunaboat Association (ATA) papers has been made available at the San Diego State University regional history archive. It is a vast and untapped source of data for analysis of these issues, and a large segment of the trainee's time in this project (before October 1988) has been allocated to surveying, organizing, copying, and analyzing a significant part of this archival data base. Additional time in data collection and analysis should result in a fresh scholarly analysis of the tuna industry of California and the impact of commercial, diplomatic, and scientific policies over a long period critical to the industry and to parts of the state's economy.

Because of the volume of materials that became available in the ATA archives, I delayed until December 1987 complementary research in University of Washington archives and special collections. Also, Professor Robert Friedheim of the University of Southern California (USC) generously shared data on Law of the Sea negotiations and issues. The Seattle collections, especially the Donald McKernan Papers, permitted us to accomplish another major objective of the research, to examine both trade and Law of the Sea policies as they emerged in the 1950s–1980s. They offered a perspective on how fisheries interests of the United States were accounted for and treated in American policy making and on how U.S. policy officials and elective officials assessed the importance of Japanese competition in Pacific waters and in U.S. markets. Augmenting this research is a segment of the work centered on sources in the Hawaii State Archives and the Honolulu library collections, illustrating the early development of South Pacific tuna fisheries in the context of U.S.-Japanese economic and diplomatic relations. It was not originally expected that the Hawaii research, initially centered on POFI and tuna development in the Eastern and Central Pacific, would produce materials on this theme. But because such material became available, it has become possible through these research investigations to link our new findings systematically with what Professor Cicin-Sain is currently investigating in relation to contemporary issues of the Exclusive Economic Zone and

fisheries agreements producing a new ocean management regime in the South Pacific.

Previous studies of the history since the 1930s of oceanographic science in relation both to marine resources management and to policy had largely been concerned with the Atlantic Ocean region (e.g., Schlee, 1973). I (Scheiber, 1986, 1987, 1988a) and my student and associate in early Sea Grant–sponsored research (McEvoy, 1986; McEvoy and Scheiber, 1984) have provided the first comparable studies of Pacific research, both California-based and regionally, and the relationship of science to policy and diplomatic issues. The present project goes beyond these previous studies, moreover, by linking the history of ocean science and diplomacy in a systematic way to emerging commercial rivalries and complementarities (Scheiber, 1988a, 1988b). Invaluable studies of international Law of the Sea negotiations by Friedheim and others (e.g., Akaha, 1985; Friedheim et al., 1984) and related factors in domestic policy process are available in the literature, but the present project is producing data on the fisheries that provide a significantly enhanced perspective on Law of the Sea development. In following months, it is expected that the computerized set of references at USC on Japanese positions on ocean-law issues, offered by Professor Friedheim for use in this regard, will be consulted further so as to complement available Japanese publications and documents. Meanwhile, I continue to plan additional analysis of material in the U.S. government archives and to conduct such interviews with participants, beyond those already completed, as can be arranged. Age and ill health are factors that work against interview arrangements with those who participated in negotiations 30–40 years ago. Interviews with two leading Japanese policy officials, with U.S. fisheries scientists, and with other figures in the policy process have been satisfactorily concluded and offered some valuable additional insights for analysis of the central bodies of data and documentation.

I have presented research findings from this and predecessor projects in a variety of forums and publications. The present project has contributed to a monograph in the field of science and Law of the Sea (Scheiber, 1987) and to an invited paper to the International Congress on History of Oceanography at Hamburg (1987). Additionally, I have served as an adviser to the Roger Revelle Oral History Project, conducted by a former doctoral student, Sara Sharp; presented a report with John Dwyer on request of the U.S. Congress, Office of Technology Assessment (restricted until 1989); prepared materials for course use in Boalt Hall School of Law, on environmental law and on technology and law; and chaired an effort that resulted in an international conference at Boalt, under auspices of the University of California and Sea Grant, on Japan, the United States, and Pacific resources since 1945, involving prominent Japanese and North American scholars, (April 1988). I also have served in this period as reviewer on marine affairs proposals for the National Science Foundation and as coordinator for marine affairs of the California Sea Grant College Program.

Cooperating Organizations

Boalt Hall School of Law, University of California, Berkeley, Sho Sato Fund for Japanese-American Legal Research
Canadian Department of External Affairs
Gerald R. Ford Presidential Library, Ann Arbor, Michigan
Hawaii State Archives
Hawaii State Library
Hoover Institution, Stanford, California
San Diego State University
Scripps Institution of Oceanography Archives
Tokyo University, Department of International Relations
U.S. National Archives
University of Hawaii Library
University of Michigan, Bentley Library
University of Southern California
University of Washington Library and Archives

References

Akaha, T. 1985. *Japan in global ocean politics.* University of Hawaii Press and Law of the Sea Institute, Honolulu.

Borden, W. S. 1984. *The Pacific alliance: U.S. foreign policy and Japanese trade recovery, 1947–55.* University of Wisconsin Press, Madison.

Friedheim, R., editor. 1984. *Japan and the new ocean regime.* Westview Press, Boulder, Colorado.

McEvoy, A. 1986. *The fisherman's problem: Ecology and law in the California fisheries.* Cambridge University Press, Cambridge, England.

McEvoy, A., and H. N. Scheiber. 1984. Scientists, entrepreneurs, and the policy process. *J. of Econ. Hist.*, 44:393–406.

Scheiber, H. N. 1986. Pacific Ocean resources, science, and Law of the Sea: Wilbert Chapman and the Pacific fisheries, 1945–70. *Ecol. Law Q.*, 13:381–534.

Scheiber, H. N. 1987. Wilbert Chapman and the revolution in U.S. Pacific Ocean science and policy, 1945–51. In *Nature to its greatest extent: Western science in the Pacific.* P. Rehbock, and I. MacLeod, eds. University of Hawaii Press, Honolulu. pp. 223–244.

Scheiber, H. N. 1988a. U.S. Pacific fishery research since 1945: Geopolitics, oceanography, and marine fisheries expansion. Invited paper, presented at the 4th International Congress on History of Oceanography, Hamburg, West Germany, September 1987.

Scheiber, H. N. 1988b. Origins of the abstention doctrine in international law: Japan–U.S. relations and the Pacific fisheries, 1945–70. *Ecol. Law Q.* 16(1)23–99. University of California Press, Berkeley.

Schlee, S. 1973. *Edge of an unfamiliar world: A history of oceanography.* Cornell University Press, Ithaca, New York.

Publications

Scheiber, H. N. 1986. Pacific Ocean resources, science, and Law of the Sea: Wilbert Chapman and the Pacific fisheries, 1945–70. *Ecol. Law Q.* 13:381–534.

Scheiber, H. N. 1987. Wilbert Chapman and the revolution in U.S.

Pacific Ocean science and policy, 1945-51. In *Nature to its greatest extent: Western science in the Pacific.* P. Rehbock and I. MacLeod, eds. University of Hawaii Press, Honolulu. pp. 223–244.
Scheiber, H. N. In press. U.S. Pacific fishery research since 1945: Geopolitics, oceanography, and marine fisheries expansion. *Deutsch. Hydrogr. Zeit.*
Scheiber, H. N. 1989. Origins of the absention doctrine in international law: Japan–U.S. relations and the Pacific fisheries, 1945-70. *Ecol. Law Q.* 16(1)23–99. University of California Press, Berkeley.
Scheiber, H. N. and J. Dwyer. 1988. *Sovereignty and Technology.* Office of Technology Assessment, Washington, D.C.

Lectures

Scheiber, H. N. Ocean resources and Law of the Sea. CLIO Project, institute for teachers, University of California, Berkeley, August 1986.
Scheiber, H. N. Environmental law. Coyote Point Museum Lecture Series, San Mateo, California, October 1986.
Scheiber, H. N. Marine policy research and public history. Presented at the History Department, Bowling Green State University, Bowling Green, Ohio, September 1987.
Scheiber, H. N. Geopolitics, oceanography and marine fisheries expansion–U.S. Pacific research since 1945. Paper presented at 4th International Congress on History of Oceanography, Hamburg, West Germany, September 1987.
Scheiber, H. N. Economic interdependence and ocean resources: The Pacific fisheries, 1945–70. Paper presented at the Economic History Association, Detroit, Michigan, September 1988.
Scheiber, H. N. Technology and legal development. Panel presentation to University of California Intercampus Economic History Group, Conference on Technology and Economic Development, San Francisco, October 1988.

Rapid Response

Abaone Larval Transplants as an Approach to Stock Enhancement

University of California, San Diego
R/NP-1-15D
Project Initiated: October 1, 1986
Project Completed: December 31, 1987

Mia J. Tegner, Earl E. Ebert, and David O. Parker

In areas where larval supply is a major factor limiting population size, the release of advanced-stage larvae may be a highly cost-effective approach to stock enhancement. The Palos Verdes Peninsula of Los Angeles County once supported a substantial population of pink abalone (*Haliotis corrugata*) but this disappeared when the Palos Verdes kelp forests were lost because of a combination of pollution and the El Niño of 1957–1959. The kelp forest and other species of abalones have recovered to various degrees, but pink abalones remain rare, probably because of limited larval dispersal from distant populations (Tegner and Butler, 1985). Thus, Palos Verdes offers an opportunity for an unambiguous test of larval releases as an approach to stock enhancement.

A technique was developed to transport advanced-stage abalone larvae, out of water, at the California Department of Fish and Game's Marine Resources Laboratory, Granite Canyon. This technique uses Nitex (nylon) screening for the larvae. The larvae are "sandwiched" between two layers of seawater-moistened polyfoam and supplied with pure oxygen. Relatively large numbers of larvae (~900/cm^2) can be transported in relatively small coolers. Laboratory trials revealed survivorship that exceeded 90% for the 48-hour test duration.

Three sites at Palos Verdes, ranging in water motion from a semiprotected cove to an exposed point, were selected, and a census was done at each site before the larvae were released. The criteria for site selection included depth (≥6 m); appropriate juvenile habitat; a *Macrocystis* forest with a history of stability and minimal potential for transition to an urchin barren; the presence of lower-standing kelps and foliose red algae; and, in order to minimize the potential for disruption by fishermen, a location within the portion of the peninsula closed to all abalone fishing.

Larvae were released between July and November 1986. An estimated 4.3 million pink abalone larvae were cultured at Granite Canyon and shipped to Long Beach out of water. Divers deployed the larvae from plastic bags directly over marked sites. In quiet water, the larvae sank very quickly in a limited area: moderate surge conditions appeared to spread the larvae over a few meters. Fish were attracted to the activity of the divers, but we have not observed any feeding behavior that suggests that the fish were responding to the larvae.

The Whale Observatory site, an open coast location, was evaluated in May, 7 months after the completion of larval release. Given a pink abalone growth rate of 18–19 mm in the first year (E. Ebert, unpublished data), we expected juveniles resulting from the experiment to be about 7–12 mm. We found 10 juvenile pink abalones in this size category in 104 m^2 of suitable juvenile abalone habitat inside our marked release site and one within 1 m of the line. No juveniles of this size were found in an additional 178 m^2 around the release site, suggesting that dispersal of the competent-to-settle larvae was minimal.

Lunada Bay, a semiprotected site, was evaluated 1 year after the release of 336,430 larvae. The inner-bay site (which received one-third of the larvae) had silted over and was no longer suitable habitat. Three juvenile pink abalones (8–12.5 mm) were found in the edge of the kelp forest quadrat even though this site had been overgrazed by sea urchins. No abalones in this size category were found in 119 m^2 of suitable juvenile habitat located outside the release sites.

Resort Point, an exposed coast location subject to considerable surge, was evaluated in September 1987, 10 months after the release of 912,000 viable larvae along three transect lines. Sixteen juvenile pink abalones, 13–21 mm, were found in 132 m^2 within 1 m^2 of the transect lines, a highly significant (t test, p = .0012) increase in density from the prerelease surveys. Two abalones in this size category were found about 10 m downswell of the release lines, probably a reflection of the surge in this habitat; an additional 116 m^2 of suitable juvenile habitat away from the lines had no pink abalones in this size category.

A total of 624 m^2 of suitable juvenile habitat was surveyed north and south (>50 m) of the release sites to look for natural settlement of pink abalone. Only one animal less than 25 mm was found, strong evidence that the animals found in the study sites resulted from our larval releases.

Although the results were encouraging, higher recovery rates would be desirable. Now that we know that larval dispersal is minimal under the conditions of our experiments, we are planning a series of small-scale experiments with red abalone larvae at Point Loma. Red abalones, considered hardier in culture and more resilient to fishing pressure, may be a better species for larval releases as an approach to abalone stock enhancement.

Cooperating Organizations
California Department of Fish and Game

References
Tegner, M. J. and R. A. Butler. 1985.

Drift tube study of the dispersal potential of green abalone (*Haliotis fulgens*) larvae in the southern California Bight: Implications for recovery of depleted populations. *Mar. Ecol. Prog. Ser.* 26:73–84.

Ocean Policy Program

Roger Revelle

University of California, San Diego
R/NP-1-16B
Project Initiated: May 1, 1987
Project Completed: November 30, 1987

Two questions are central to this preliminary research on establishing an ocean policy program at the University of California, San Diego (UCSD): Should more researchers be trained in ocean policy? and How do we best assist their study? Specifically, our research is aimed toward developing a structure and curriculum for such a program.

The major goal of our project has been to assess the resources, interests, and strategies available to support an expanded program in ocean policy at UCSD. We realize that to take advantage of the tremendous ocean policy expertise here in Southern California, we must be able to address questions posed by scientists, governmental agencies, industry leaders, politicians, and students at both the graduate and undergraduate level.

Despite the enormous importance of ocean-related issues to the State of California, there has yet to emerge a significant center for building expertise on these issues within California. To the extent that this project will bring us closer to building an ocean policy program it will contribute in a major way toward providing the tools that are demanded when any major state issue in ocean policy is addressed.

Our first step has been to conduct a survey of other marine policy programs by writing to selected experts in this field at other schools and in key agency positions. We are interested in learning from them about the structure of their programs, their curriculum, and what they think are important challenges in establishing any new ocean policy program. We then intend to consolidate responses from this survey with an assessment of available resources at UCSD and formulate a recommended model, or set of alternative models, for an ocean policy program at UCSD.

The research was initiated with the hiring of a Sea Grant trainee to carry out the survey and conduct the assessment of local resources. We began with meetings in June 1987. A survey has been drafted and mailed to 13 recipients who have had extensive experience with ocean policy programs. The trainee has met with and interviewed more than half a dozen of the important actors in ocean policy in and around UCSD. He has retrieved from the Scripps Institution of Oceanography (SIO) archives a 1983 report, prepared for the Sloan Foundation, that contains a complete review of factors relating to the formation of a Pacific Ocean Forum fellowship program, as well as a design and budget for that program.

Now in process are analysis of survey responses, assessment of ocean policy resources at UCSD and SIO (including library resources, existing courses, and faculty interest), assessment of other local resources (San Diego State University, University of San Diego, Southwest Fisheries Center, and the U.S. Navy), and estimation of budget.

Study of alternative program models include (1) upgrading the existing undergraduate program in marine policy and environmental studies under the Program for Science, Technology and Public Affairs, UCSD; (2) creating a graduate-level program (master of marine affairs) within the new School of International Relations and Pacific Studies; (3) establishing a postdoctoral fellowship program and/or a professional fellowship program at SIO; and (4) building a semiautonomous ocean policy institute that might interact more freely with other expert resources in Southern California. We anticipate completion of this report near the end of 1987.

Cooperating Organizations
Environmental Science and Policy Institute, San Diego

California and Ocean Governance: Toward a Long-Term Strategy

University of California, Santa Barbara
R/NP-1-16D
Project Initiated: September 15, 1987
Project Completed: March 31, 1988

Biliana Cicin-Sain

The major purpose of this project was to examine the state's actual and potential role in planning for the development and protection of Exclusive Economic Zone (EEZ) resources. The study examined, in broad terms, the state's economic and environmental concerns in offshore resources and the existing capacity of state agencies to deal with the technical and policy issues raised by EEZ development.

The following tasks were completed:

(1) a review of the literature on the history of ocean and coastal management initiatives in California in the past twenty years,

(2) a review of the literature on the current organization of state government vis-à-vis ocean and coastal resources,

(3) a set of personal interviews wtih state officials concerned with ocean and coastal management in March 1988,

(4) a review of the literature on various uses in the EEZ offshore California, (including offshore oil development, fisheries, recreation, marine transportation, waste disposal, ocean mining, aquaculture, and military activity) both in terms of their current status, as well as projections on trends until the year 2000 (whenever available),

(5) a review of the planning activities regarding the EEZ recently undertaken by a number of states (Oregon, Washington, North Carolina, Hawaii) and by a number of state organizations (such as the Coastal States Organization and the National Governors' Conference).

Initially, our intention was to prepare a brief report (about 50 pages in length) aimed at policy makers and the informed public in the state reviewing the likely expansion in development offshore California, the current organization and activities of the relevant state agencies, the experience of other states, and possible future alternative courses of action for California. Two factors have caused us to alter our initial plan in the direction of a more in-depth treatment of the subject matter and a longer report: (1) the complexity of the subject matter and the large body of data that we were able to gather on the major study questions and (2) funding we have received from the National Coastal Resources, Research, and Development Institute to conduct a related study, which will examine the same types of issues as the current study, but in greater depth and in the context of all four Pacific Coast states (Alaska, Oregon, Washington, and California). Hence, we have been able to devote additional staff resources to the project.

Temporal Change of Deep-sea Hydrothermal Vent Communities

University of California, San Diego
Scripps Institution of Oceanography
R/NP-1-17B
Project Initiated: January 1, 1985
Project Completed: December 31, 1988

Robert R. Hessler

One of the most interesting facets of deep-sea hydrothermal vent environments is their instability. Mere decades are thought to separate the initial flow through newly opened fissures and the cessation of circulation brought about by cooling of the heat source. During this period, both the spatial pattern of vent openings within the field and the water quality may alter. This dynamic milieu must be a strong stimulus for community change. However, until recently, little direct biological evidence has been available, largely because few vent fields have been visited more than once.

To date, the best documentation of temporal change in vent communities comes from the Rose Garden vent field on the Galapagos spreading center (00°48.247′N, 86°13.478′W) at a depth of 2470 m in the eastern tropical Pacific. This was one of the first vents to be visited by biologists in 1979, at which time it was heavily photographed. From these observations, a general description of the community was made (Hessler and Smithey, 1983).

In 1985, we visited this vent field again, only to find that the community had undergone substantial change (Hessler et al., 1988). Several species that were dominant in 1979 were now present in very low numbers. Populations of other members of the fauna had grown considerably. Two hypotheses to explain these changes are plausible: (1) this was a response to changes in physical conditions at the vent: and (2) the changes were biologically engendered, being the result of colonization and competition.

Although neither of these hypotheses could be eliminated, a few facts narrowed their constraints. There were no obvious changes in the distribution of vent openings, water temperature, or water chemistry. Unfortunately, no one has ever succeeded in measuring the flow rate of water from Galapagos-type vents, so we cannot know whether flux had diminished. There was some indication that sulfide, which is all-important as an energy source, was being filtered out by mussels, making it unavailable to the tube worms (Johnson et al., 1988). If mussels were so effective at filtering emerging vent water, it could be that they also effectively filtered out all the particulate matter that would be the food source for the near-field suspension feeders. Thus, the steady growth of the mussel population could explain the diminution of other species populations.

These observations decisively showed that vent communuties do indeed change with incredible rapidity by deep-sea standards and convinced us of the value of following this and other selected vent fields through time. We would learn considerable basic biology of this newly discovered biotope, and we would obtain information that would be useful in gauging the potential response of these communities to human disturbance such as mining. Accordingly, in the spring of 1988, biologists made another visit to the Galapagos Spreading Center. Funding from Sea Grant made it possible for me to participate and resurvey vent fields in order to document change. On this cruise, we not only revisited Rose Garden but also surveyed two other vent fields: Musselbed, which had not been seen since 1979, and New Vent, which was surveyed photographically but not visited in 1985.

My approach was much as before, relying largely on photographic documentation. On the 1985 cruise, the main survey system was ANGUS, a camera array towed by a surface vessel: its photographs allowed us to map the community at Rose Garden. In 1988, all photography was conducted from the submarine Alvin. Four systems were used: a vertically oriented mapping camera (substituting for the ANGUS system), obliquely oriented survey cameras for routinely monitoring Alvin's movements over the bottom, a closeup stereo camera to document fine-scale distributions and smaller animals, and hand-held cameras for anecdotal documentation.

Our effort yielded thousands of photographs. Even with the disappointments about to be mentioned, it should be possible to remap Rose Garden and to map New Vent for the first time. The 1985 ANGUS surveys are also available for mapping New Vent as seen at that time. Thus, we are in a position to make detailed comparisons of changes at those vents during the last 3 years. This will not be possible at Musselbed, because the documentation from 1979 is inadequate and because the great topographic relief precludes photographic surveys with Alvin.

Unfortunately, a variety of problems reduced the quality of some of our data sets below what we would have liked. Some of the high-altitude surveys were degraded by inadequate light and turbidity. Photographs obtained with the obliquely oriented cameras were often underexposed because Alvin cruised at too high an altitude. The stereo camera broke down and required a part for which we had no replacement. Problems with Alvin's strobe lights caused underexposure of most of the photographs obtained

with hand-held cameras.

Although it is too early to enumerate differences from previous visits in detail, it is possible to discuss our overall impressions. In view of the large changes witnessed between 1979 and 1985, we expected to see further dramatic changes after another 3 years. In actual fact, this did not happen. Rose Garden, which is best known, looked much as it had when last seen. Clumps of bivalves and giant tube worms were found in the same pattern as before, and there were no major changes in scavengers or in the surrounding vent-field suspension feeders. We could detect some changes in relative abundance. For example, even though their distribution had not changed, there were fewer giant tube worms in their clumps. Similarly, Musselbed and New Vent looked much as they had at earlier times. Once again, we noticed no change in the distribution of vent openings or water chemistry.

These new observations strengthen the hypothesis that biological factors have driven the development of communities on the Galapagos Spreading Center. The composition of the community at Rose Garden in 1985 and 1988 appears to be relatively stable. By contrast, in 1979, it was obviously in rapid flux, suggesting that it was a young community. If true, it would be expected that other vent-field communities that look like that of Rose Garden in 1985 and 1988 would also be stable. The lack of major changes at Musselbed and New Vent, which have that composition, bears this out.

Closer scrutiny of our 1988 data will undoubtedly add many refinements to our observations, including possible perception of subtle but significant changes. These observations also indicate the value of further visits to the Galapagos vents to see if the 1985–1988 condition is truly a climax community and to witness what happens when vent flow finally diminishes.

Cooperating Organizations
National Geographic Society
National Science Foundation
Moss Landing Marine Laboratory
Rutgers University
University of California, Davis.
University of California, Santa Barbara

References

Hessler, R. R., and W. M. Smithey, Jr. 1983. The distribution and community structure of megafauna at the Galapagos Rift hydrothermal vents. In *Hydrothermal Processes at Seafloor Spreading Centers.* P. A. Rona, K. Bostrom, L. Laubier, and K. L. Smith, Jr., eds. NATO Conference Series. IV: Marine Sciences. Plenum, New York. pp. 735–770.

Hessler, R. R., W. M. Smithey, Jr., M. A. Boudrias, C. H. Keller, R. A. Lutz and J. J. Childress. 1988. Temporal change in megafauna at the Rose Garden hydrothermal vent. *Deep-Sea Res.* 35:1681–1709.

Johnson, K. S., J. J. Childress, R. R. Hessler, C. M. Sakamoto-Arnold, and C. L. Beehler. 1988. Chemical and biological interactions in the Rose Garden hydrothermal vent field. *Deep-Sea Res.* 35:1723–1744.

Continuing Projects

Sea Grant Extension Program

The California Sea Grant Extension Program (SGEP) is organized into four major program areas: Marine Fisheries, Seafood Technology, Coastal Resource Management, and Aquaculture.

The area of marine fisheries remains the most extensive area within the SGEP. The seven marine advisors conduct significant marine fisheries projects in their regions. The advisors receive support from the marine fisheries specialist, a broad-based university research program, several fisheries agencies, and industry. Also, the Sea Grant Extension seafood technology program and parts of the coastal resource management program are closely linked to marine fisheries efforts.

The overall objectives of the marine fisheries program are to (1) increase knowledge about marine fisheries and fisheries issues and research among industry, management agencies, and interested citizens; (2) evaluate and develop supplemental fisheries to help fishermen diversify their operations; (3) improve fisheries enhancement practices and techniques; and (4) improve the efficiency and safety of marine fishing operations by applying research-based information.

SGEP staff have divided the marine fisheries program into five subprogram areas based on needs assessment and available resources: Fisheries Efficiency and Safety; Fisheries Utilization and Management; Fisheries Enhancement; Fisheries Education; and Fishing Gear Technology.

Improving fishermen's safety practices, energy efficiency, and the application of environmental data in fishing operations are the major emphases of this subprogram. Implementation methods used include workshops, publications, videotapes, consultations, and research demonstrations.

Recent state legislation made funds available for low-interest loans and an educational/research program to increase fuel efficiency in the commercial fishing fleet.

During 1987–88, continued and increased project funding was obtained from the California Energy Extension Service. A Canadian propeller optimization computer program was rewritten and used by marine advisors. At-sea tests of hydrodynamically efficient trawl doors showed an average fuel savings of 6%. This finding was supported by our flume-tank tests in Denmark. In comparison to a standard shrimp trawl, an expanded mesh shrimp trawl on at-sea tests showed fuel savings, speed increase, reduced warp tension, and equal shrimp catches.

Long-term tests continued on copper-based, self-polishing, antifouling paints. Five workshops on the loan program were held, and evaluation of the first 17 loan-program participants estimated their fuel savings at $98,000 for the life of the technology. An in-depth description and evaluation of a project was prepared and is being used to initiate similar programs in other states. An article was prepared and submitted to *National Fisherman*. A project research assistant received his M.S. in Naval Architecture at UC Berkeley, reporting the project results for his thesis. The project was featured in a *Marine Technology Journal* paper as a model for Sea Grant technology transfer.

Sea-surface temperature charts increase efficiency by saving time and fuel. During 1987–88, sea-surface temperature charts were posted by most marine advisors at local ports. Use increased dramatically, especially in Monterey Bay.

Included in the marine fisheries program is the Vietnamese safety project. Lack of safety equipment, poor safety practices, and the inability to use English to call the U.S. Coast Guard have resulted in the loss of lives and vessels of Vietnamese-speaking commercial fishermen in California.

During 1987–88, a Vietnamese-language safety video-tape we produced was used by fishermen and fisheries associations in California, on the U.S. Gulf Coast, and in Canada. Awareness of marine safety equipment has increased among the Vietnamese-speaking fishermen in California. Coast Guard radio operators have been able to understand emergency calls from Vietnamese refugee fishermen.

The fisheries utilization and management subprogram was established to provide technical and research information on newly developed and established fisheries, including data on life history of the harvested species and on marketing, utilization, and management. Also, new management techniques can be taught to employees at established fisheries.

Until the early 1980s sheep crab (a by-product of the halibut set-gill-net fishery) was an underutilized species in south central California and was often regarded as a nuisance to net fishermen. A recent marketing effort by fishermen and processors has resulted in increased demand for sheep crab and a significant jump in landings.

During 1987–88, a literature search was completed. A SGEP program representative started research on the sheep crab life history and fishery.

Angel shark landings have expanded in the Santa Barbara Channel in recent years, and the objective of this project is to obtain biological information needed for the management of this fishery. During 1987–88, the project was completed. Research data were consolidated for the California Department of Fish and Game (CDFG) and industry to develop a

fisheries management plan starting in 1989. Meetings were organized to develop a management plan. Tagging studies will be continued by CDFG.

Most Dungeness crabs are landed in northern California during the first three to four weeks of the season when prices are lowest. Approximately four weeks after the season starts, the crab supply drops dramatically and prices rise. Holding a part of the catch for four to eight weeks might result in higher prices to offset the cost of crab enclosures, feeding costs, and losses due to mortality. During 1987–88, the project was completed. The three-week holding experiment showed that crabs did best when not fed and when held at densities between 13.7 to 17.1 pounds per cubic foot (no mortality; 4.4% weight loss). A report was completed and distributed to industry.

One project was designed in 1986 to determine angler perception of underutilized fish species and fishing opportunities on commercial passenger fishing vessels and to apply this knowledge in test-marketing projects. During 1987–88, angler and vessel owner sampling was completed, and results were distributed to the California passenger fishing vessel industry and National Marine Fisheries Service (NMFS). Funding was obtained for continuation of the project. A test-marketing project targeted at 500 corporations and a project to demonstrate onboard refrigeration were started.

Limited entry and individual transferable quota (ITQ) schemes have been implemented in some fisheries. To inform the fishing industry about innovative management techniques, nine months of research was completed examining early effects of New Zealand's ITQ system on industry. A research report was submitted to New Zealand's Ministry of Agriculture and Fisheries, and results were presented to the Pacific Fisheries Management Council's limited-entry committee and the North Pacific Fisheries Management Council's Future of Groundfish committee. A CDFG advisory committee developed a limited-entry plan for the rapidly expanding sea urchin fishery.

Needs exist to enhance fisheries by habitat-improvement projects and improved fishery management techniques, to train public enhancement groups in new rearing and habitat techniques, and to improve communication among public enhancement groups. More long-term anadromous fish resource information is needed, and conflicts between sport and commercial fishermen need to be lessened through public involvement in enhancement projects.

Habitat restoration and resource enhancement have become popular and effective methods of restoring salmonid resources. The objective of this project is to improve the knowledge and skills of public groups involved in fisheries enhancement projects. During 1987–88, one marine advisor completed a sabbatical leave visiting salmon enhancement projects in the Pacific Northwest and British Columbia. Another advisor tagged, scale-sampled, and released over 100 adult steelhead to evaluate the smolt-release program of the Monterey Bay Salmon and Trout Project. Also, he and a SGEP researcher collected blood samples of Coho salmon and steelhead to study optimization of smolt-release programs. In another project, CDFG forest inspectors were trained on salmonid habitat needs and restoration techniques, and a spreadsheet program was developed for enhancement groups to use to monitor growth, feeding, and temperature. Several marine advisors served on the planning committee for the California Salmon, Steelhead, and Trout Federation Conference.

In another project, SGEP researchers work to improve fisheries enhancement in the Smith River system and to develop a long-term historic data base. Waste water discharges from the new Pelican Bay Prison may significantly affect Smith River salmonids. This long-term project will be extended ten years to document any changes.

Industry leaders, teachers, agency representatives, and the general public need updated information about fisheries. The objectives of one project are to train teachers and others who provide marine information to the public and to increase the awareness of marine educators about coastal fisheries issues. During 1987–88, the classroom aquarium incubation project begun in Humboldt County schools, was implemented in dozens of schools as far south as Monterey. Marine advisors provided teacher training and industry cooperators for the project. Other accomplishments included wetlands training for 4-H leaders and fisheries training for schoolteachers in the San Francisco Bay Area, curriculum development for the State Salmon and Steelhead Advisory Committee, hosting a meeting on lowering obstacles to 4-H marine education programs, and co-sponsoring the National Marine Educators Association meeting in Santa Cruz.

A new tuna treaty has been ratified to help resolve the problem of South Pacific nations seizing U.S. tuna vessels. Fishermen need to understand and abide by the regulations of this treaty. In 1987–88, the project was completed. An educational videotape and companion booklet were produced and distributed to the tuna fleet and Pacific island governments through NMFS.

The 1971 CDFG publication *California's Living Marine Resources and Their Utilization*, needs updating. During 1987–88, two meetings were organized with NMFS, CDFG, UC, and fisheries representatives to develop strategies for accomplishing this.

The fishing gear technology subprogram involves the development of techniques for supplemental fisheries, improving new gear technology, and reducing gear conflicts.

Continued restriction of inshore

netting of croaker, rockfish, and halibut has forced fishermen to seek alternative gear. During 1987–88, the Vietnamese Fishermen's Association of America (VFAA) and the Coastal Fisheries Foundation were assisted in securing funds for alternative gear projects. Also, the fishing industry needs independent information about innovations in fishing gear. The emphasis of this project is on gear that increases economic efficiency. During 1987–88, over 150 trawlers participated in a statewide series of trawling workshops and individual consultations that emphasized fuel-saving technologies.

The overall objectives of the Coastal Resource Management program area are to (1) promote wise use, management, and conservation of coastal resources; (2) reduce conflicts among user groups; and (3) improve communication and cooperation among resource managers, key leaders, and resource users. On the basis of identified needs, resources, and priorities, the Coastal Resource Management program area is divided into three subprogram areas: Multiple Use of Resources; Port, Harbor, and Marina Management; and Coastal Resources Education.

Multiple use of resources creates conflicts, and resolving conflicts among user groups is a priority of the National Sea Grant College Program (NSGCP). This subprogram seeks to encourage communication among groups and to disseminate applicable research results.

The objectives of a project on offshore oil and gas development are to identify the impacts of oil development on ocean resources and on marine-related industries; improve communications among fishermen, offshore operators, and resource managers; and reduce conflicts between the fishing and oil industries.

During 1987–1988, a marine advisor assisted the Southern California Lobster Fishermen's Association in resolving potential conflicts between geophysical survey operators and commercial fishermen. Potential loss of 4,000 spot prawn traps and 50 halibut nets was avoided by negotiations. One marine advisor hosted meetings between fishermen and representatives of offshore oil and geophysical companies. Another marine advisor gave presentations on offshore oil/fisheries communications and conflict-resolution methods to West Coast legislators and the Washington State Legislature's Joint Select Committee on Marine and Ocean Resources; this advisor also completed a report on offshore oil/fisheries conflict resolution for the Joint Select Committee.

SGEP personnel hosted three subcommittees of the Washington Sea Grant Ocean Resources Assessment Project (ORAP). Experiences on oil development from south central California were provided by the marine advisor, commercial fishermen, oil industry representatives, county planners, and university researchers.

Also, two workshops were conducted at which an underwater Minerals Management Service videotape of north coast fishing grounds was shown to fishermen, students, and members of the public. Editing and distribution of the tape to interested industry members and the public were arranged. Copies of the *Oil and Gas Project Newsletter for Fishermen and Offshore Operators* were distributed each month. The use of a newsletter to reduce conflicts was introduced to Washington State fishermen, the oil industry, and government agencies in 1988.

SGEP staff also helped coastal communities to mitigate the effects of offshore oil/gas development to offset these impacts, the state government and coastal counties have established various funds using oil revenues. During 1986–87, one marine advisor served as the vice-chair of the Humboldt County Outer Continental Shelf (OCS) Advisory Committee and a member of the Fisheries Subcommittee. The subcommittee developed a comprehensive list of lease-sale stipulations as mitigation for fisheries impacts. They also identified lease-sale tracts with particularly sensitive habitats and recommended their deletion. The County Board of Supervisors adopted these recommendations and submitted them to Minerals Management Service and the Governor's office as comments on the Lease Sale 91 Draft Environmental Impact Statement.

Another marine advisor worked with the Joint Offshore Oil and Fishing Industry Liaison office, the Mediation Institute, and the Governor's Office of Environmental Affairs to develop an organizational structure for an industry/agency committee to oversee the operations of a Local Fisheries Impact Program. The program, funded by California's share of oil revenues from offshore oil drilling, helps offset some past impacts on the fishing industry from offshore oil development. A SGEP seafood technology specialist, marine fisheries specialist, and marine advisor were appointed by the California Secretary of Environmental Affairs to serve as members of the Fisheries Impact Program Technical Advisory Committee.

A different project focuses on the constantly increasing commercial and residential coastal development, which degrades or destroys California's wetlands.

During 1987–1988, a marine advisor served on the Monterey Bay Task Force and chaired the program subcommittee for the State of the Bay Conference. He also helped develop a Department of Health Services study on pesticides and trace metals in Monterey Bay fish and provided information on the fate of specific compounds in the environment and on pesticide use patterns for selected crops, and served on the Elkhorn Slough National Estuarine Research Reserve (ESNERR) Advisory Committee and its research subcommittee. He assisted in designing the Reserve Master Plan

and provided information to the National Oceanic and Atmospheric Administration (NOAA) and the Office of Coastal Resource Management (OCRM).

Efforts in port, harbor, and marina management are focused on improving waterfront managers' ability to provide the best service to boat owners and to waterfront communities.

The objectives of the coastal waterfront managers survey are to profile California coastal boating facilities and managers and to identify the education and research needs of port, harbor, and marina managers as the foundation for an education and research program. During 1987–1988, marine advisors completed an analysis and report on the survey of 206 California coastal waterfront managers. Topics of five seminars at the annual conference of the California Association of Harbor Masters and Port Captains were chosen on the basis of survey results, which were presented to the Pacific Coast Congress of Harbor Masters and Port Managers and to the Southern California Marine Association Lease Information Exchange Committee. Waterfront managers, consultants, government agencies, and university faculty have requested the report for use in planning and decision making.

The coastal resources education project seeks to supply research-based information to California citizens to increase their understanding of our coastal resources and ocean and coastal zone multiple-use issues.

The objectives of the marine education training project are to train teachers, 4-H leaders, and others who provide marine education to the public and to increase the awareness of marine educators about coastal resources and their use and management.

In 1987–1988, a marine advisor and a program representative conducted a pilot marine education program. Ten demonstrations and field trips were tested and evaluated; participants included two elementary school teachers (one bilingual) and 35 fifth/sixth grade students (the majority Hispanic). The teachers were trained in marine education methods and coastal resource topics.

The NSGCP has identified marine plastic pollution education as a national priority. This project seeks to increase awareness of the pollution problem among vessel operators and harbor managers and teach them how to reduce the problem. U.S. laws require ocean-going vessels to return plastics and other debris to shore. Marine terminal operators must provide adequate reception and disposal facilities.

In 1988, a marine advisor participated in the National Sea Grant workshop "Oceans of Plastic," visited the marine debris-collection project organized by Oregon Sea Grant, and provided lists of fishermen's and waterfront managers' associations to the U.S. Coast Guard for notification of rulemaking. A fisheries specialist and two marine advisors featured the rulemaking process and the debris problem in their newsletters. Information on the problem and the rulemaking process was presented to meetings of the Pacific Coast Congress of Harbor Masters and Port Managers and to the San Diego Sportfishing Association.

The major goals of the seafood technology project are to (1) improve the quality of seafood available to consumers; (2) increase the use of modern technology in seafood processing, marketing, and waste management; and (3) increase industry and public knowledge of seafood nutrition, safety, and handling.

Workshops and short courses on canning technology, food-processing sanitation, statistical quality control, food microbiology, and freezing technology were provided for industry personnel. Workshops and short courses for specific audiences included "Caviar Processing and Quality Control" for quality control technicians working in China; "Food Plant Sanitation" for students, faculty, and food industry personnel at the Universidad Iberoamericana, México, D.F.; "Food Plant Sanitation" for Dominican Republic food professionals; and "Fish and Shellfish Handling, Processing, and Quality Control" for Pakistani fisheries and aquaculture professionals. Also, marine advisors and specialists assisted the California Seafood Institute in completing a final draft of the Seafood Menu and Advertising Guidelines.

With Saltonstall-Kennedy funding, two marine advisors conducted handling and storage studies to evaluate the effect of on-board handling on fresh and frozen albacore quality and shelf life. Round, bled, and dressed albacore were held in ice, brine, brine-coil, and blast freezer systems on commercial fishing vessels, and then transferred to UC Davis for one-month (fresh) and 12-month (frozen) quality and shelf-life studies.

In research on handling and packaging sport-caught tuna, Nationwide Marketing donated two vacuum-packaging machines and plastic film, and Mar-Cal Seafood agreed to cooperate in a demonstration and assessment of vacuum packaging and freezing as an alternative to custom canning or standard freezing of sport-caught albacore. A marine advisor completed 75% of the on-board data collection; he compared the efficiency of slush ice and wet gunny sack cooling of sport-caught fish. In a follow-up to a 1986–87 educational program, 200 anglers were surveyed for changes in practices and attitudes. Of 97 respondents, 10% took home more of their catch, 18% took better care of their catch on the way home, and 16% consumed more of their catch.

Seafood and wine promotions were expanded to include Tomales Bay Oyster Growers who served and displayed their product at the 1987 Sonoma County Wine Auction. Seafood-wine promotional activities have demonstrated the value of the "concurrent marketing concept." The Sonoma County marketing team, initiated by the Sonoma/Marin

Counties marine advisor, received the Cooperative Extension award for "Distinguished Service as an Outstanding Creative Team" for their work in encouraging commodity producers to focus on marketing activity.

SGEP personnel provided training to food professionals and Cooperative Extension staff on seafood safety, safe handling and home preservation of seafood, and new developments in seafood technology.

In the area of seafood waste management, a marine advisor completed a sea-urchin-waste application demonstration project on a Tomales Bay dairy; a compost demonstration project using cow manure and sea urchin shells and viscera; and two demonstration projects to hydrolyze seafood-processing wastes, using squid at the Pt. Judith Cooperative and sole at the Town Dock Fish Company. During his sabbatic leave, this marine advisor completed a survey to determine the amount and type of liquid fish and kelp being used by farmers in New England, the Great Lakes area, the upper Midwest, the mid Atlantic states, and the Pacific Coast states; investigated the effectiveness of using marine products as fertilizer or livestock feed additives; visited established and potential manufacturers of liquid fish and kelp; and documented current research on using fish and kelp as a fertilizer or livestock feed additive.

Aquaculture is a substantial and important activity in California. Advisory activities focus on problems associated with the established oyster industry and on research information about the developing technologies for the production of new aquaculture species.

Offshore mariculture has increased off Santa Barbara County, and some of the mariculture operations interfere with commercial fishing operations. A marine advisor and a program representative documented specific conflicts. Assisted by representatives from the fishing and mariculture industries, they conducted a tour of offshore mariculture operations for representatives of CDFG managers and Wildlife Protection Branch supervisors. After the tour, SGEP hosted an information-exchange meeting to review specific problems, industry suggestions for resolving them, CDFG mariculture regulations, and ways to improve communications between the industries.

During 1987–1988, work on shellfish sanitation has been limited. Elkhorn Slough, once among California's major shellfish production areas, has been restricted from direct harvesting of shellfish since 1969, because of coliform pollution. Changes in the watershed suggest that water quality has improved. One marine advisor organized a meeting of the Department of Health Services (DHS), Monterey County Health Department, the ESNERR, State Mussel Watch, and university researchers. A monthly water-quality monitoring program was established, DHS and county activities were coordinated, and water quality of commercial leases were included in monthly sampling.

Another aquaculture project seeks to increase interested residents' access to sources of sound information on aquaculture technology and management and to improve efficiency in advising them.

One marine advisor developed a fact sheet "Making the Fish Farming Decision in Southern California." It listed sources of information on cultural practices, economic analysis, regulations and registration, and UC and government agency assistance. Cooperative Extension offices throughout California have used it effectively to handle public service calls.

Another marine advisor reported the following aquaculture activities: meeting with representatives of FDA, DHS, and a local oyster company to present sanitation data collected during last year by Coast Oyster Company and analyzed by SGEP office; presentation made to officials of local timber company and a private firm interested in starting an abalone farm in Humboldt County; review of hatchery techniques in California and Japan; information on trout culture provided to representatives of Blue Lake Rancheria; publication of observations on Japanese aquaculture; assistance to a trout operation in Mendocino in applying for federal drought-relief funds; and many activities related to salmon enhancement program.

Research on new aquaculture species is being conducted within both university and industry laboratories. Improved culture and production technologies are also being developed by both the public and private sectors. For example, recent university and industry research on developing mass culture methods for the blue mussel, *Mytilus edulis*, have been highly successful, and shellfish growers in California, Oregon, and Washington have taken a renewed interest in the blue mussel as a primary product or as a supplemental species to diversify their oyster, clam, or abalone operations. One limiting factor in the mussel culture system has been juvenile grow-out, when the recently settled mussels are moved from the hatchery to the grow-out area. Specific questions relate to optimum substrate for growth and survival, density of seeding, optimum seeding size, holding and feeding of seed, and predation control.

Cooperating Organizations

Ab Lab
Abalone Farms, Inc.
The Abalone Shop
Abalone Unlimited, Inc.
Alaska Commercial Fisheries Entry Commission
Alaska Sea Grant College Program
American Fisheries Society
American Fisheries Society, Humboldt Chapter
American Tunaboat Association
Anderson's Boat Service
Anthony's Seafood Grottoes
Anthony's Seafood Market
Aquaculture Digest
Arcata Union

Assemblyman Sam Farr's Office
Association of Monterey Bay Governments
Atlantic Offshore Fishermen's Association
Audubon Society
Battelle Memorial Institute
Benech Biological, Inc.
Berkeley Marina
Blount Seafood, Warren, Rhode Island
Blue Lake Rancheria
Bob Morrel Enterprises, Inc.
Bodega Bay Fisheries Marketing Association
Bordynsky (Joe), C.P.A.
Brandon King Seafoods
Brookings Fishermen's Marketing Association
Brookings Fishermen's Wives
CAT Diesel Engines
California Abalone Association
California Aquaculture Association
California Association of Harbor Masters and Port Captains
California Coastal Commission
California Coastal Operators Group
California Conservation Corps
California Cooperative Fisheries Research Unit
California Department of Boating and Waterways
California Department of Corrections
California Department of Environmental Affairs
California Department of Fish and Game
California Department of Food and Agriculture
California Department of Forestry
California Department of Health Services
California Department of Motor Vehicles
California Department of Parks and Recreation
California Energy Extension Service
California Farm Bureau
California Gillnetter's Association
California Marine Parks and Harbors Association
California Marine and Navigation Conference
California Maritime Academy
California Office of Environmental Affairs
California Office of Planning and Research
California Salmon, Steelhead, and Trout Restoration Federation
California Sea Farms
California Seafood Institute
California Shellfish Company
California State Coastal Conservancy
California State Lands Commission
California State University, Chico
California State University, Hayward
California Urchin Divers Association
Canadian Department of Fisheries and Oceans, British Columbia
Canadian Department of Fisheries and Oceans, Newfoundland Region
Candlestick Park State Recreation Area
Captain's Ice Company
Carmel River Steelhead Association
Castle Rock Fisheries
Caterpillar Tractor, Inc.
Central California Council of Divings Clubs, Inc.
Central Coast Hook and Line Association
Central Coast Seafoods
Channel Island National Marine Sanctuary
Channel Island National Park Service
Chesapeake Fish Company
Chetco STEP, Inc.
Circuit Rider Productions
Cloudburst Fishing Company
Coast Chandlery, Santa Barbara
Coast Marine Industrial Supply
Coast Oyster Company
Coastal Fisheries Foundation
College of the Redwoods
Commercial Fishermen of Santa Barbara, Inc.
Commercial Fishermen's Wives of Humboldt
Congressman Doug Bosco's Office
Continental Shelf Associates
Crescent City Fishermen's Wives
Crescent City Harbor District
Crescent City Parks and Recreation
Cuesta College
The Cultured Abalone
Curry County Fishermen's Association
Dana Point Marina
Dana Wharf Sportfishing
Danish Institute of Fisheries Technology
Del Ackerlund Farms, Valleyn, Nebraska
Del Norte County Board of Supervisors
Del Norte Fishermen's Marketing Association
Del Norte Triplicate
Delaware Sea Grant Program
Devoe Paint Company
Diesel Comp Fuel Systems
ECOMAR, Inc.
EG and G Oceanographic Services
ERG Pacific
East Bay Regional Park District
Eel River Restoration Project
El Granada Elementary School
Elk Valley Rancheria
Elkhorn Slough Foundation
Elkhorn Slough National Estuarine Research Reserve
Elkhorn Yacht Club
Energy, Mines and Resources, Canada
Eureka Fisheries, Inc.
Eureka Times-Standard
F/V *Abrigo*
F/V *Apollo*
F/V Arnie P.
F/V *Blue Pacific*
F/V *Caito Brothers*
F/V *Christopher*
F/V *Cat Special*
F/V *Clara G*
F/V *Cortez*
F/V *Donna J*
F/V *Flying Fish*
F/V *Fred Holmes*
F/V *Freelance*
F/V *Ginnie C II*
F/V *Gus D.*
F/V *Holiday*
F/V *Janus*
F/V *Marion W.*
F/V *Miss Rickie*
F/V *Mr. Bill*
F/V *New LoAn*
F/V *New Ray Ann*
F/V *Prowler*
F/V *Salty Lady*
F/V *St. Christopher*
F/V Sea
F/V *Sea Otter*
F/V *Shogun*
F/V *Tradewind*
"Fish Phone"
The Fish Tail Market
Fisheries Protection Institute
Fisheries and Oil Industries Joint Committee
Fisheries and Oil Industries Liaison Office
The Fisherman
Fishermen's Cooperative Association
Fishermen's Landing
Fishermen's Marketing Association, Inc.
Fishermen's Union—I.C.W.U. Local 33
Five-Cities-Times-Press Recorder
Florida Sea Grant College Program
Friends of Del Norte
Friends of Garcia River
Friends of the Sea Otter
Geophysical Services, Inc.
Golden Gate Fishermen's Association
Golden Gate National Recreation Area
Great Barrier Reef Authority
Greater Los Angeles Council of Divers
Gregorio Aquatech
Gulf of the Farallones National Marine Sanctuary
Half Moon Bay Fishermen's Association
Half Moon Bay Public Library
Hawaii Sea Grant College Program
Hog Island Oyster Company
Hopkins Marine Station
Howorth & Associates, Santa Barbara
Humboldt Bay Fisheries Association
Humboldt Bay Harbor, Recreation, and Conservation District
Humboldt County Board of Supervisors
Humboldt County Farm Bureau
Humboldt County Planning Department
Humboldt Fish Action Council
Humboldt Fishermen's Marketing

Association
Humboldt State University
ICF, Inc.
International Institute for Transportation and Ocean Policy Studies
Island Packers
J.A.C. Creative Foods
J.J. Camillo Seafood Company
Johnson Oyster Company
Joint Committee on Fisheries and Aquaculture (Senator Barry Keene, Chair)
Kamilche Sea Farms, Washington
King Harbor, City of Redondo Beach
LMR Resources, Inc.
Living Marine Resources
The Log
Long Beach Marine Bureau
Long Marine Laboratory
Los Angeles County Department of Beaches and Harbors
MCTV
Mar-Cal Seafood
Marin Fish and Game Advisory Committee
Marine Associations Council of California
Marina City Club
Marine Mammal Commission
Massachusetts Division of Marine Fisheries
Massachusetts Institute of Technology Sea Grant College Program
Max Machinery Company
Mediation Institute
Mendocino County Department of Environmental Health
Mendocino Fish Advisory Committee
Meyer Resources, Inc.
Michael Brandman Associates
Miller-Rellim Redwood Company
Mixner/Scott, Inc.
Mobil Oil Company
Monterey Bay Anadromous Fish Advisory Committee
Monterey Bay Aquarium
Monterey Bay Salmon and Trout Project
Monterey County Health Department
Monterey Harbor, City of Monterey
Monterey Peninsula Flycasters
Morro Bay Commercial Fishermen's Association
Morro Bay Harbor Department
Moss Landing Commercial Fishermen's Association
Moss Landing Harbor District
Moss Landing Marine Laboratories
Moss Landing Marine Supply
NOYO Women for Fisheries
National Coastal Resources Institute
National Fisheries Institute
National Fisherman Magazine
National Marine Educator's Association
National Marine Fisheries Service
National Marine Manufacturers Association
National Ocean Service
National Shellfisheries Association
National Weather Service
Nationwide Marketing
The Nature Conservancy
Navy Post Graduate School
New Growth Forestry Services
New York Sea Grant College Program
New Zealand Federation of Commercial Fishermen
New Zealand Ministry of Agriculture and Fisheries
Nor Cal Truck Specialties
North Carolina Sea Grant College Program
North Pacific Fisheries Management Council
Northern California Indian Development Council
Northern California Marine Association
Ocean Fare Sales and Marketing
Oceanic Society
Oceanside City Harbor District
Office of Coastal Zone Resource Management
Orange County Marine Institute
Orange County Sheriff-Coroner Department
Oregon Coastal Operators Association
Oregon Department of Fish and Wildlife
Oregon Land Conservation and Development Commission
Oregon Pacific Salmon Ranch
Oregon Sea Grant College Program
Orion Elementary School
Pacific Choice Seafoods
Pacific Coast Fishermen's Wives Coalition
Pacific Coast Congress of Harbor Masters and Port Managers
Pacific Coast Federation of Fishermen's Associations, Inc.
Pacific Coast Fishermen's Wives Coalition
Pacific Coast Guides Association
Pacific Coast Oyster Growers Association
Pacific Fishery Management Council
Pacific Fishing Magazine
Pacific Mariculture Inc.
Pacific Seafood Industries
Pacific Trawl Company
Palladini Fish Company
Pillar Point Harbor
Pitchometer Propellers
Point Loma Seafoods
Point St. George Fisheries
Port of Brookings
Port of Gold Beach
Port of Long Beach
Port of Los Angeles
Port of Oxnard
Port of San Diego
Port San Luis Commercial Fishermen and Boat Owners Association
Prairie Creek Fish Hatchery
Producers' Seafoods
Provincial Fisheries Department, British Columbia
Qualman Oyster Farm
Queensland Fisheries Management Authority
Race Lagoon Mussels, Washington
Radio KCRE
Radio KEKA
Radio KFLI
Radio KOMY
Radio KPOD
Radio KURY
Radtke, Hans
Redwood Community Action Agency
Redwood National Park
Rhode Island Sea Grant College Program
Rogue River Guides Association
Rowdy Creek Fish Hatchery
Rural Human Services
Russian River Committee
Salmon Trollers Marketing Association of Fort Bragg
Salmon Unlimited
Salty Lady Sportfishing
San Diego County Department of Agriculture
San Diego County Planning Department
San Diego County Public Health Department
San Diego Log
San Diego Sportfishing Association
San Diego State University
San Diego Union
San Francisco Bay Fisherman's Association
San Francisco Bay National Wildlife Refuge
San Jose State University
San Lorenzo River Steelheaders
San Marcos Unified School District
San Pedro Fish Market
San Luis Obispo County Planning Department
Santa Barbara City Planning Department
Santa Barbara City Schools
Santa Barbara Commercial Fishermen
Santa Barbara County Board of Supervisors
Santa Barbara County Fish and Game Commission
Santa Barbara County Resource Management Department
Santa Barbara Harbor Department
Santa Barbara Museum of Natural History
Santa Barbara News Press
Santa Barbara Sea Center
Santa Cruz Commercial Fishermen's Association
Santa Cruz County Planning

Department
Santa Cruz Port District
Science Applications International
Scow Enterprises, Fremont, Minnesota
Scripps Hydraulics Laboratory
Sea Farms West
Sea Ventures
Seafood Specialties
Share Fishermen of New Zealand
Shellfish Institute of North America
Shoreline Marketing Associates
Six Rivers National Forest
Smith River Anglers Association
Society of American Foresters (Sonoma/Mendocino Chapter)
Sonoma County Grape Growers
Southcoast Sport Fishermen's Association
Southern California Lobstermen's Association
Southern California Marina Association
Southland Farmer's Market Association
Sportfishing Association of California
Sportfishing Institute
State Fish Company
Sun Bulletin, Morro Bay
Sunland Seafoods, Inc.
Sydney University, Department of Veterinary Science
TV Station KRCR
Telegraph Hill Neighborhood Center
Texas A&M University Sea Grant College Program
Town Duck Seafood, Galilee, Rhode Island
Trans National Agronomic, Grand Rapids, Michigan
Trinidad Fishermen's Marketing Association
United Anglers, Inc.
United States Tuna Foundation
United States Tunaboat Association
U.S. Army Corps of Engineers
U.S. Coast Guard
U.S. Coast Guard Auxiliary
U.S. Department of Agriculture
U.S. Department of Interior
U.S. Department of Interior Minerals Management Service
U.S. Fish and Wildlife Service
U.S. Food and Drug Administration
U.S. Forest Service
U.S. General Accounting Office
U.S. Small Business Administration
U.S. Soil Conservation Service
United Anglers of California
United Anglers, Inc.
University of California, Davis, Bodega Marine Laboratory
University of California, Berkeley Department of Naval Architecture
University of California, San Diego, Scripps Aquarium-Museum
University of Rhode Island, Marine Advisory Service
University of Rhode Island, Master Gardner Coordinator
University of Southern California Sea Grant Program
University of Washington, Manchester Laboratory
Ventura County Fishermen's Association
Ventura Harbor District
Vietnamese Fishermen's Association of America
Vietnamese Pacific Fishermen's Association
W. R. Merry Seafood Company
Washington Department of Ecology
Washington Department of Fisheries
Washington Department of Game
Washington Dungeness Crab Fishermen's Association
Washington Sea Grant College Program
Washington State Legislature
West Coast Fisheries Development Foundation
Western Fishboat Owners Association
Western Oil and Gas Association
Westlog, Inc.
Western Association for the Valuation of Ecosystems (WAVE)
Wisconsin Department of Natural Resources
Women's Fisheries Network

Publications

Alverson, F. 1988. Purse seine fishermen's guide to the South Pacific Tuna Treaty. 18-page guide to videotape. (See Dewees, C.M. and L. T. Johnson.)

California Department of Fish and Game, United States Department of Commerce (National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Region) and University of California (Cooperative Extension, Sea Grant Extension Program). 1987. *Marine Sportfish Identification.* 164 pp.

Chang-Lee, M. V., R. J. Price, and L. E. Lampila. 1989. Effect of processing on proximate compostion and mineral content of sea cucumbers (*Parastichopus* spp.). *J. Food Sci.* 54:567.

Dewees, C. M., and Grover, J. 1987. Technique of making fish illustration 25: More on gyotaku. *Environmental Biology of Fishes* 18(4):313–317.

Dewees, C. M., and G. R. Hawkes. 1988. Technical innovation in the Pacific Coast trawl fishery: The effects of fishermen's characteristics and perceptions on adoption behavior. *Human Organization* 47:224–234.

Dewees, C. M. 1988. New Zealand's ITQ system. In *Proceedings from national conference on matching capital to resources in the fish harvesting industry.* Atlantic Offshore Fishermen's Association and University of Rhode Island. pp. 77–88.

Dewees, C. M., and L. T. Johnson. 1988. South Pacific Tuna Treaty. 29-minute videotape.

Johnson, L. 1987. Making the fish farming decision in Southern California. Sea Grant Extension Program, University of California, Davis. UCSGEP 87–7. 2 pp.

Johnson, L. T. 1987. Sport fish handling project introduction, Summer/Fall 1987. Fact Sheet 87–2. San Diego County Cooperative Extension. 1 p.

Johnson, L., and R. Price. 1988. Vacuum packaged and frozen tuna. San Diego County Sea Grant Extension Program Fact Sheet 88–1. 2 pp.

Johnson, L., and C. Ryan. 1988. In press. California coastal waterfront managers survey. California Sea Grant Extension Program.

Melvin, E. 1988. Fish quality: A fisherman's responsibility. *Vietnamese Fishermen's Association of America Newsletter.*

Price, R. 1987. Surimi—A new ingredient for the meat processor. *Proceedings of the 1987 Meat Processing Conference.* University of California, Cooperative Extension.

Price, R. J. 1988. Paralytic shellfish poisoning and red tides. Sea Grant Extension Program, Cooperative Extension, University of California, Davis. UCSGEP 88–1. 2 pp. Revision.

Price, R. J., S. S. O. Hung, F. S. Conte, and E. M. Strange. 1989. Processing yields and proximate composition of cultured white sturgeon (*Acipenser transmontanus*). *J. Food Sci.* 54(1)216–217.

Price, R. J., and P. Tom. 1988. Smoking fish. Sea Grant Extension Program, Cooperative Extension, University of California, Davis. UCSGEP 88–2. 2 pp.

Radtke, H., C. M. Dewees, and F. J. Smith. 1987. The fishing industry and Pacific coastal communities: Understanding the assessment of economic impacts. University of California, Sea Grant Extension, Marine Advisory Program Publication, UCSGMAP–87–1. 10 pp.

Richards, J. B. 1988. Conflicts between the oil and gas industry and the commercial fishing industry on the

south-central California coast. In: *Washington State Legislature, Report of the Joint Select Committee on Marine and Ocean Resources.*
Ryan, C., E. Melvin, and N. Duc. 1987. Safety and Survival at Sea; An educational tape for Vietnamese Fishermen. Videotape. In Vietnamese.
Strange, E. M., and Dewees, C. M. 1987. Notes in bottles clue to sea currents. *Outdoor California* 48(4):16–18.
Tom, P., and R. J. Price. 1987. Resource: Health advisories on California fish. *Nutrition Perspectives* 11(5):10. H. Swenerton, ed. University of California, Cooperative Extension.
Tom, P., and H. Swenerton. Resource: Paralytic shellfish poisoning and red tides. *Nutrition Perspectives* 13(6):4. H. Swenerton, ed. University of California, Cooperative Extension.
Toole, C. L. 1988. Report of a study trip to Japan. I. Molluscan aquaculture and fisheries. 68 pp. II. Salmonid aquaculture and fisheries. 37 pp. III. Fisheries education and extension activities. 22 pp. California Sea Grant College Program Working paper P–T–48.
Toole, C. L., R. A. Barnhart, and C. P. Onuf. 1987. Habitat suitability index models: Juvenile English sole. U.S. Fish and Wildlife Service Biological Report 82 (10.133). 27 pp.
Wyatt, B. 1987. Which fish?—What wine? Foldout pamphlet by Sonoma County Grape Growers.
Wyatt, B. 1987. Fuel efficiency loans handled by Sea Grant. *The Fishermen's News* 43(5):4.

Communications

The Communications Office of the California Sea Grant College Program plays an essential role in disseminating information about the activities and accomplishments of the program and in promoting communication among a variety of audiences involved in marine resource management, conservation, and development.

Located at the Program's administrative headquarters at UC San Diego, the Communications Office has these major objectives:

1. To inform a wide spectrum of audiences about the mission and activities of the state and national Sea Grant programs;
2. To inform public, industry, scientific, legislative, and other audiences about findings arising from Sea Grant-sponsored research;
3. To educate a wide spectrum of audiences about state, national, and international marine-resource issues;
4. To assist and support the information dissemination activities of program management.

Background

The California Sea Grant College Program is the largest in the national network. The state it serves has 15 coastal counties stretched along a thousand-mile coastline. Eighty percent of California's population, or some 20 million people, are estimated to live within 30 miles of this coast, and the population continues to grow rapidly. Given the concentration of people along the coast and the wealth of resources in the Pacific Ocean, marine-related issues are extremely important within the state. These issues are reflected in the research, education, and advisory activities of the program, and range from the health and viability of California's fisheries to the vulnerability of the coast to erosion and the effects of offshore oil development.

California Sea Grant supports strong, sophisticated research in marine science and technology. In 1987-88, for example, the Communications Office reported on 38 Sea Grant-sponsored research projects at seven of the nine campuses of the University of California and five campuses of the California State University system. The projects fell into the general areas of Coastal Resources, Aquaculture, Fisheries, New Marine Products, Ocean Engineering, and Marine Affairs.

The program director is the chief spokesperson for the program. His information dissemination and public relations activities are varied and range from Congressional testimony to presenting student awards.

Publications Rationale

Because the potential of Sea Grant research and other activities is not met unless the results generated get into appropriate hands, the work of our principal investigators is reported at different levels for different audiences. Most of our efforts are directed to reaching leaders in the legislature, academia, government agencies, and industry.

Three publications form the foundation of our publications efforts. The first is an annual *Program Directory* of currently funded projects. This publication provides a general program overview plus a guide to current Sea Grant-sponsored work throughout the state.

A second publication which we consider fundamental is our *Summary*. It is perhaps our major public information product, and we plan to publish it annually. Written for the educated layman, the *Summary* allows us to report noteworthy accomplishments in all of our spheres of activity and to develop a number of themes that set program activities in a different or larger context.

The 1988 *Summary Report* was organized around the theme *California Sea Grant: Coastal Ocean Science and Technology*. This report presented a comprehensive assessment of the benefits accruing to California from Sea Grant-sponsored activities. In addition to describing program successes in, for example, identifying and treating salmon diseases, contributing to the establishment of sturgeon culture, protecting the state's endangered wetlands, and exploring sea life for natural products, the report gave a comprehensive listing of cooperating organizations over a five-year period and another listing of conferences and symposia sponsored by the program. The report was written for the educated layman and has been widely disseminated within the state. This five-year perspective on program accomplishment is required by the Resources Agency of the State of California.

The 1987 *Summary*, titled *Sea Grant: A National Resource for Marine Research and Education*, examined the role of academic research and graduate education in determining our nation's economic competitiveness.

A third core publication is the *Biennial Report of Completed Projects*, in which each principal investigator reports his or her progress in language appropriate for peers. It forms an essential historical record of program accomplishments, including publications and results, and thus represents an important document in terms of both program accountability and dissemination of scientific and technical results.

Additional publications reflect areas of special interest or emphasis within the program. Those produced in the 1986-88 period are listed at the end of this report.

Dissemination

It is the policy of California Sea Grant to encourage researchers to publish their results in professional journals. The Publications Office attempts to monitor the publications

activity of our researchers as one important measure of program productivity and to disseminate all published materials to appropriate parties.

In addition to our standard distribution procedures, each title is added to a widely distributed publications list (issued twice yearly by the Publications Office) as well as to *Sea Grant Abstracts*, which is distributed nationally.

In 1987-88, the Information Specialist distributed reprints of 100 journal articles and papers from published conference proceedings. In addition, she distributed publications in the California Sea Grant series (produced by this department) and miscellaneous publications in a number of categories for a total of 137 different items, or 15,763 pieces. Addition of publication announcements, press releases, and awards announcements brought the number of pieces distributed to 34,293.

The Information Specialist not only handles initial distribution of publications, but also maintains files of reprints and books from which to fill both specific and general requests for information. In 1987-88, there were 1,209 "unsolicited" requests for information or publications (i.e., not directly generated by our own publications announcements), bringing the total number of pieces distributed to 41,690.

Public Information and Special Projects

The Communications Office is responsible for media relations and public information activities, such as issuing press releases. It also produces a number of miscellaneous products on an annual basis. These include portions of the institutional proposal, brochures, certificates and plaques, acknowledgement and reprint guidelines, and the Call for Annual Reports. The Office also provides assistance to the Program Manager on special projects as requested.

Sea Grant Reference Series

Amidei, Rosemary. 1987. *Sea Grant: A National Resource for Marine Research and Education.* R-CSGCP-021. 36 pages, 16 figures.

Amidei, Rosemary. 1987. *University of California: Ocean Science and Technology.* Brochure. 4 pages, 7 figures.

Amidei, Rosemary, editor. 1988. *California Sea Grant: Coastal Ocean Science and Technology*–A Report to the Resources Agency Sea Grant Advisory Panel. Five year report, 1982-1987. No. R-CSGCP-023. 64 pages, 25 figures.

California Sea Grant College Program. 1986. *California Sea Grant Biennial Report, 1982-84*, A Report on the California Sea Grant College Program for October 1, 1982 to September 30, 1984. No. R-CSGCP-020. 266 pages, 66 figures.

California Sea Grant College Program. 1987. *California Sea Grant 1987-88 Program Directory.* No. R-CSGCP-022. 28 pages, 5 figures.

California Sea Grant College Program. 1988. *California Sea Grant Biennial Report of Completed Projects, 1984-86.* No. R-CSGCP-024. 154 pages, 39 figures, 30 tables.

California Sea Grant College Program. 1988. *California Sea Grant 1988-89 Program Directory.* No. R-CSGCP-025. 27 pages, 6 figures.

Sea Grant Technical Series

Isabella A. Abbott, editor. 1988. *Taxonomy of Economic Seaweeds*, With reference to some Pacific and Caribbean Species, Volume II. Results of an international workshop sponsored by the California Sea Grant College Program and the Institute of Oceanology of the Academia Sinica of the People's Republic of China in cooperation with the Pacific Sea Grant College Programs of Alaska, Hawaii, Oregon, and Washington and hosted by the Institute of Oceanology in Qingdao, September 22 to 25, 1986. No. T-CSGCP-018. 265 pages, 268 figures, 4 tables.

Amidei, Rosemary, editor. 1986. *Rockfish: A Focus for Research*? Proceedings of a California Sea Grant Workshop, April 4, 1986, University of California, Davis. No. T-CSGCP-015. 71 pages.

Amidei, Rosemary, editor. 1987. *Educating Fisheries Managers:* Proceedings of a California Sea Grant Workshop, April 3, 1986, University of California, Davis. No. T-CSGCP-016. 44 pages.

Amidei, Rosemary, editor. 1988. *West Coast Mollusc Culture: A Present and Future Perspective.* Proceedings of a California Sea Grant Workshop in cooperation with the Pacific Sea Grant College Program, July 9-10, 1987, University of California, Berkeley. No. T-CSGCP-017. 87 pages, 8 figures, 2 tables.

Zedler, Joy B. and Christopher S. Nordby. 1987. *The Ecology of Tijuana Estuary: An Estuarine Profile.* Produced jointly with the U.S. Fish and Wildlife Service. 104 pages, 70 figures, 30 tables.

Other

Christopher Toole. 1988. *Report of a Study Trip to Japan.* Working Paper No. P-T-48. Three volumes. 127 pp.

Education

Sea Grant's commitment to education and training activities in the marine sciences remains evident in the projects it supports for students at all levels, as well as for the general public.

The Trainee Program

Virtually all of the research projects supported by California Sea Grant involve at least one graduate student trainee. During their training, students conduct independent marine research while working alongside University scientists and engineers in demanding and stimulating research environments. This new talent will be responsible for maintaining America's scientific and technological leadership in coming years. More than 600 students have been involved in the California Sea Grant trainee program since its inception.

In 1986-87, 50 Sea Grant trainees worked with project leaders at 9 California universities and colleges. Another 62 trainees were supported in 1987-88. Most of these students worked on or completed graduate degrees during their traineeships.

Isaacs Scholarship

The $10,000 Isaacs Scholarship is awarded each year to a California high school senior who has entered a project in the State Science Fair. The award recognizes excellence and encourages students to continue their marine education at California colleges and universities. James T. Randerson, the winner in 1987, looked at how a submerged horizontal cylinder, such as a pipeline, influences ocean waves. Jim, a graduate of Point Loma High School, is now enrolled at Stanford University.

Russell Scott Shapiro, 1988 winner, studied changes in the sediment and general environment of the south branch of Scripps Submarine Canyon off La Jolla. Russell, a graduate of University City High School in San Diego, is now a student at Humboldt State University.

Marine Sciences for the Public

The overall goal of the California Sea Grant Education project in 1987-88, to increase the public awareness of current marine-related issues in the context of their social, political, economic, and Pacific ramifications, was primarily addressed through several teacher training workshops, with an emphasis on the incorporation of existing materials into the educational system. In addition to these workshops, the project supported curriculum design and distribution, lecture series coordinated with workshops, an international symposium, and curriculum development symposia at a week-long meeting of the National Marine Educators Association.

At the southernmost site, the Scripps Aquarium-Museum, University of California, San Diego, hosted an international symposium on the water cycle. Co-sponsored by the Canadian Consulate, the Water Cycle Symposium, held January 23, 1988, featured noted experts from both the United States and Canada. Following opening remarks by Roger Revelle of Scripps Institution of Oceanography, seven speakers addressed the various steps in the water cycle, from air-sea interactions through acid rain, down rivers to the sea, and finally to the wetlands. The symposium proceedings were transcribed and edited, and now await only printing costs to see them through to completion. Several corporate and public utility sponsors are being sought to cover these costs.

In addition to an international symposium, the Scripps Aquarium-Museum sponsored several teacher's workshops. In addition to the annual workshops, "Living in a Watery World, sections I & II" (for K-6th grade teachers), which were enjoyed by close to 160 teachers, new workshops were designed, entitled "Coastal Marine Life of San Diego" and "Kelp Forest Ecology" (for 4-8 grade teachers).

Finally, the UCSD group aided in the development and design of a ten chapter high school curriculum and workbook in oceanography for the San Diego City and County Schools.

A two-day workshop at the UC Santa Barbara site was coordinated by the UC Santa Cruz group. This workshop, co- sponsored by the Channel Islands National Marine Sanctuary, and entitled "Humans—Just Another Species in the Marine Ecosystem?", met with the approval of forty local teachers, grades 3 to 12. On Friday, April 29, the participants were treated to a boat tour of the Channel Islands. On Saturday activities included discussions of current research topics, such as mariculture, effects of pollutants, and seabirds as biological indicators. Each teacher received a packet of background materials as well as resource lists and grade-level activities to take back to their classrooms. Continuing education credit was offered through University of California Extension.

At Moss Landing Marine Lab four teachers workshops were held, with over eighty teachers participating. These workshops, taught by Kathleen Dickey, were entitled "Deep Sea Research." They were held over a two-day period, May 21-22, with teachers from four surrounding counties attending. Continuing education credit was offered through San Jose State University.

In addition to teacher's workshops, Moss Landing lent support to the curriculum planning and development sessions at the annual conference of the National Marine Educator's Association (NMEA) (see further description below). Berndt Wursig and Greg Caillet made presentations at the

conference and then aided in the design of curriculum plans by the conference attendees. Kathleen Dickey, in association with the Monterey Bay Aquarium, planned and coordinated a teacher's workshop and the curriculum development sessions at the NMEA conference.

At UC Santa Cruz, ninety teachers attended four workshops coordinated with a major theme of the NMEA conference, "Communication in Marine Systems." Berndt Wursig and graduate student Carl Schilt spoke on dolphin communication and fish schooling, respectively. Teachers received a packet containing sample lesson plans and background materials. Continuing education credit was offered through University of California Extension. The UC Santa Cruz—Sea Grant Education project also co-sponsored a lecture series (with the Santa Cruz City Museum) entitled "Nautical Neighbors: A Marine Mammal Lecture Series." Teachers attending "Communication" workshops were requested to attend these public lectures held on Wednesday evenings during the month of February.

In addition to four teacher's workshops at Santa Cruz, a lecture series, and coordination of the two workshops at UC Santa Barbara, the UC Santa Cruz group, co-hosted with the Monterey Bay Aquarium and the Oceanic Society, the 1988 NMEA conference, held on the UC Santa Cruz campus, July 19-22, 1988. This conference was attended by more than 300 marine science teachers from throughout the United States. Local California teachers from workshops at Santa Cruz, Moss Landing, and the Monterey Bay Aquarium served as facilitators at curriculum development symposia and workshops on "Marine Animal Communication," "Deep Sea Discoveries," and "Threats to the Marine Environment." An outstanding speakers' roster included Eugenie Clark on "Sea Monsters and Deep Sea Sharks," Steven Webster on "Bridging the Gap Between Education and Science," and Sam Hinton on "Songs of the Sea."

At the northernmost site, the Telonicher Marine Laboratory, Humboldt State University, a two-day, workshop held on sequential Saturdays dealt with a "Holistic Approach to Marine Science Education—A Bay Environment." The primary focus was on low-cost, hands-on activities for students in the classroom, at the beach, or at a marine lab. The workshops considered the history and ecology of the Humboldt Bay ecosystem, in addition to ways of integrating music and mathematics into marine science. Each of the twelve participants received an impressive 200-page curriculum/activity guide. Finally, 100 of these activity guides were distributed to schools or County Education Curriculum Resource Centers in six northern California counties, as well as several Pacific coast marine labs.

Overall, the five-campus California Sea Grant Education project reached over 380 California primary and secondary teachers through 19 workshops/symposia, through sponsorship of lecture series and an international symposium, and through the activities at the annual meeting of the National Marine Educator's Association.

Cooperating Organizations

Año Nuevo State Park
Channel Islands National Marine Sanctuary
Elkhorn Slough National Estuarine Research Reserve
Institute of Marine Sciences, University of California, Santa Cruz
Long Marine Lab, University of California, Santa Cruz
Marine Science Institute, University of California, Santa Barbara
Monterey Bay Aquarium
Moss Landing Marine Lab
Natural Bridges State Park
Santa Barbara Natural History Museum
Santa Cruz City Museum
Telonicher Marine Lab, Humboldt State University
University of California, Cooperative Extension

Appendices

Officials and Administrators

Regents of the University of California

1988-1989

Regents Ex Officio

George Deukmejian
Governor of California

Leo McCarthy
Lieutenant Governor

Willie L. Brown, Jr.
Speaker of the Assembly

Bill Honig
State Superintendent of Public Instruction

Richard G. Heggie
President of the Alumni Association of the University of California

S. Sue Johnson
Vice-president of the Alumni Association of the University of California

David P. Gardner
President of the University

Appointed Regents

Roy T. Brophy
Clair W. Burgener
Yvonne Brathwaite Burke
Glenn Campbell
Edward W. Carter
Frank W. Clark, Jr.
Tirso del Junco, M.D.
Jeremiah F. Hallisey
Willis W. Harman
John F. Henning
Meredith Khachigian
Leo S. Kolligian
Vilma S. Martinez
Joseph A. Moore
Robert N. Noyce
Jacquelyn Ross
Stanley K. Scheinbaum
William French Smith
Deborah R. Thorpe
Yori Wada
Dean A. Watkins
Harold M. Williams
William A. Wilson
Jacques S. Yeager

Regents-designate

Ronald Enomoto
Sherrill Luke

Faculty Representatives

Richard W. Gable
Murray L. Schwartz
Fred N. Spiess

Officers of the Systemwide Administration

1988-1989

David P. Gardner
President of the University

William R. Frazer
Senior Vice President, Academic Affairs

Ronald W. Brady
Senior Vice President—Administration

William B. Baker
Vice President—Budget and University Relations

Kenneth Farrell
Vice President—Agriculture and Natural Resources

Cornelius L. Hopper
Vice President—Health Affairs

Officers Emeriti

Clark Kerr
President of the University, Emeritus; Professor of Business Administration, Emeritus

Charles J. Hitch
President of the University, Emeritus; Professor of Economics, Emeritus

David S. Saxon
President of the University, Emeritus; Professor of Physics, Emeritus

Daniel G. Aldrich
Chancellor, Emeritus; Professor of Ecology and Evolutionary Biology, Emeritus

Albert H. Bowker
Chancellor, Emeritus; Professor of Statistics, Emeritus

Vernon I. Cheadle
Chancellor, Emeritus; Professor of Botany, Emeritus

Ivan H. Hinderaker
Chancellor, Emeritus; Professor of Political Science, Emeritus

Dean E. McHenry
Chancellor, Emeritus; Professor of Comparative Government, Emeritus

James H. Meyer
Chancellor, Emeritus; Professor of Animal Science, Emeritus

John B. de C. M. Saunders, M.D.
Chancellor, Emeritus; University Librarian, Emeritus; Professor of Anatomy, Emeritus; Professor of History of Health Sciences, Emeritus

Robert L. Sinsheimer
Chancellor, Emeritus

Angus E. Taylor
Chancellor, Emeritus; University Provost, Emeritus; Professor of Mathematics, Emeritus

Harry R. Wellman
Vice President of the University, Emeritus; Professor of Agricultural Economics, Emeritus; Agricultural Economist, Emeritus

William P. Fretter
Vice President of the University, Emeritus; Professor of Physics, Emeritus

James B. Kendrick, Jr.
Vice President–Agriculture and Natural Resources, Emeritus; Professor, Emeritus

Thomas E. Jenkins
Vice President—Budget Plans and Relations, Emeritus

Baldwin G. Lamson, M.D.
Vice President—Financial and Business Management, Emeritus; Professor of Pathology, Emeritus

Elmo R. Morgan
Vice President—Physical Planning and Construction, Emeritus

Dorothy E. Everett
Assistant President, Emeritus

Norman H. Gross
University Auditor, Emeritus

Clinton C. Powell, M.D.
Special Assistant to the President—Health Affairs, Emeritus

Loren Furtado
Assistant Vice President, Emeritus

August Fruge
Director of the University Press, Emeritus

Ruth E. Byrne
Coordinator, Administrative Policy, Emeritus

Gloria L. Copeland
Executive Assistant to the President, Emeritus

Chancellors

Ira Michael Heyman
Chancellor at Berkeley

Theodore L. Hullar
Chancellor at Davis

Jack W. Peltason
Chancellor at Irvine

Charles E. Young
Chancellor at Los Angeles

Rosemary S. J. Schraer
Chancellor at Riverside

Richard C. Atkinson
Chancellor at San Diego

Julius R. Krevans
Chancellor at San Francisco

Barbara S. Uehling
Chancellor at Santa Barbara

Robert B. Stevens
Chancellor at Santa Cruz

Resources Agency Sea Grant Advisory Panel

Gordon K. Van Vleck, Chairman
Secretary of Resources
Resources Agency
State of California
Sacramento, California

Peter Bontadelli
Director
Department of Fish and Game
Sacramento, California

Peter Douglas
Executive Director
California Coastal Commission
San Francisco, California

William H. Ivers
Director
Department of Boating and Waterways
Sacramento, California

Donald L. Keach
Director
USC Sea Grant Program
University of Southern California
Los Angeles, California

Senator Barry Keene
State Capitol
Sacramento, California

Assemblywoman Sunny Mojonnier
State Capitol
Sacramento, California

Gerald A. Pollock
Hazard Evaluation Section
Department of Health Services
Sacramento, California

Richard Ridenhour
Dean, College of Natural Resources
Humboldt State University
Arcata, California

Robert E. Ross
Executive Director
California Fisheries and Seafood Institute
Sacramento, California

Theodore C. Smith
Technical Planning Officer
Division of Mines and Geology
Sacramento, California

Fred Spiess
Institute of Marine Resources
A-028
University of California
La Jolla, California

F. Robert Studdert
San Rafael, California

Wilbur M. Thompson
Manager
Long Beach Operations
State Lands Commission
Long Beach, California

Daniel Walsh
State Water Resources Control Board
Sacramento, California

Elmer P. Wheaton
Portola Valley, California

Institute of Marine Resources Executive Committee

William H. Fenical, Chairman
Acting Director
Institute of Marine Resources
University of California
La Jolla, California

Wallis H. Clark
Professor of Animal Science
Bodega Marine Laboratory
Bodega Bay, California

William T. Doyle
Director
Institute of Marine Sciences
University of California,
Santa Cruz

Frederick A. Eiserling
Divisional Dean—Life Sciences
College of Letters and Sciences
University of California,
Los Angeles

Edward A. Frieman
Director
Scripps Institution of Oceanography
University of California,
San Diego

Donald Heyneman
Director
UCB/UCSF Joint Medical Program
University of California,
Berkeley

James P. Kennett
Director
Marine Science Institute
University of California,
Santa Barbara

J. R. Paulling
Naval Architecture and Offshore Engineering
University of California,
Berkeley

James J. Sims
Plant Pathology
University of California,
Riverside

Grover C. Stephens
Developmental and Cell Biology
School of Biological Sciences
University of California,
Irvine

James J. Sullivan
Director
California Sea Grant College Program
University of California
La Jolla, California

Institute of Marine Resources Advisory Council

Richard E. Attiyeh
Dean
Graduate Studies and Research
University of California,
San Diego

John P. Craven
Director
The Law of the Sea Institute
University of Hawaii,
Honolulu

Murray Dailey
Director
Ocean Studies Institute
California State University,
Long Beach

Thomas B. Day
President
San Diego State University
San Diego, California

Frank D. Drake
Dean, Division of Natural Sciences
University of California,
Santa Cruz

C. M. (Tod) Ghio
Ghio Seafood Products
San Diego, California

W. F. (Zeke) Grader, Jr.
General Manager
Pacific Coast Federation of Fishermen's Associations
Sausalito, California

Theodore L. Hullar
Chancellor
University of California,
Davis

Robert W. Morse
North Falmouth, Massachusetts

Ernest L. (Roy) Perry
Retired Executive Director
Port of Los Angeles
Tacoma, Washington

David S. Potter
Santa Barbara, California

Nathan Sonenshein
RADM, USN (Retired)
Moraga, California

Roger Thomas
Sausalito, California

Gordon K. Van Vleck
Agency Secretary
Resources Agency of California
Sacramento, California

James H. Zumberge
President
University of Southern California
Los Angeles

California Sea Grant Committee

James J. Sullivan, Chairman
Director
California Sea Grant College Program
A-032
University of California
La Jolla, California 92093

John H. Crowe
Professor of Zoology
Department of Zoology
University of California
Davis, California 95616

William T. Doyle
Director
Institute of Marine Sciences
Applied Sciences Building
University of California
Santa Cruz, California 95064

Cadet H. Hand
Director Emeritus
Bodega Marine Laboratory
P.O. Box 247
Bodega Bay, California 94923

David G. Hankin
Professor
Department of Fisheries
Humboldt State University
Arcata, CA 95521

Robert W. Holmes
Professor of Biological Sciences
Department of Biological Sciences
University of California
Santa Barbara, California 93106

Donald L. Keach
Director
USC Sea Grant Program
University of Southern California
University Park
Los Angeles, California 90089-0341

Edward B. Lyke
Professor of Biological Sciences
Department of Biology
California State University
25800 Hillary Street
Hayward, California 94542

Alec D. MacCall
Acting Director
NMFS, Tiburon Fisheries Laboratory
3150 Paradise Drive
Tiburon, California 94920

Michael M. Mullin
Director
Marine Life Research Group
Scripps Institution of Oceanography
A-027
University of California, San Diego
La Jolla, California 92093

Richard J. Seymour
Research Engineer
Marine Research Division
Scripps Institution of Oceanography
A-022
University of California
La Jolla, California 92093

Joy B. Zedler
Professor of Biology
Department of Biology
San Diego State University
San Diego, California 92182-0408

Aquaculture Industry Advisory Committee

Mike Gafford
Namakan West Fisheries
Los Banos, California

Richard Glenn
Seafarms West
Carlsbad, California

George Lockwood
Hawaiian Abalone Farms
Kailua-Kona, Hawaii

John McMullen
Ab Lab
Naval Civil Engineering Lab
Port Hueneme, California

Frank Oakes
The Abalone Farm
Cayucos, California

Anthonie M. Schuur
Aquafuture, Inc.
Bakersfield, California

Hugh W. Staton
Abalone Unlimited, Inc.
Guadalupe, California

Peter Struffenegger
Sea Farms of California
Herald, California

F. Robert Studdert
San Rafael, California

Philip L. Wilson III
Managing Partner
Aquaculture Enterprises
Kailua-Kona, Hawaii

Seafood Industry Advisory Committee

Tod Ghio, Chairman
Ghio Seafood Products
San Diego, California

Joe A. Caito
Caito Fisheries, Inc.
Fort Bragg, California

Maurice Camillo
J. J. Camillo Seafood Brokerage
San Diego, California

Mike DeGarimore
Central Coast Seafoods
Morro Bay, California

Tom Elliott
TEMA, Inc.
San Francisco, California

Bill Merry
W. R. Merry Seafood Co.
Los Angeles, California

Wayne Miller
Ocean Garden Products
Anaheim, California

William Perkins
Western Fishboat Owners Association
San Diego, California

J. David Ptak
Chesapeake Fish Company
San Diego, California

Ray Swanson
Swanson Brothers Seafood
Novato, California

Nick A. Vitalich
Chesapeake Fish Company
San Diego, California