

# Sea Grant Researcher Conference

September 25, 2003  
Galveston, Texas



*Coastal Science Serving Texans*

# Sea Grant Researcher Conference

September 25, 2003 — Galveston, Texas

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# Dermo Watch: Current Status of Dermo Disease in Oysters of Three Texas Bays

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Through its range from Mexico to Maine, *Perkinsus marinus* is a major cause of mortality in eastern oysters, *Crassostrea virginica*. The parasite was first described from Louisiana oysters as *Dermocystidium marinum*, from which its common name "Dermo" is derived. Because there is no economical or feasible treatment of infected oysters, the only effective approach to control Dermo is proper management. The parasite is more prevalent at high water temperature (T) and high salinity (S) and is thus most problematic during late summer and on the seaward side of estuaries. Possible management techniques include maintaining freshwater inflows, diverting fresh water into high-salinity estuaries, early harvest of infected oysters and moving infected oysters to low salinity waters.

The Dermo Watch Program was established to more effectively manage Dermo in the Gulf of Mexico. Dermo Watch is a website ([www.blueblee.com/dermo](http://www.blueblee.com/dermo)), a monitoring program and an online community for the management of *P. marinus*. The website contains an embedded model that calculates a time to critical level (t-crit) of disease from an initial level of disease and water T and S. Thus, samples of oysters are collected and water T and S are measured. An initial weighted incidence (i-WI) of parasitism is determined using Ray's fluid thioglycollate method. A critical WI (c-WI) is set at 1.5, Water T, S, i-WI are inputs to the model and by stimulation a t-crit is determined as the number of days to reach c-WI, assuming no change in T or S.

Through a cooperative program with Texas Parks and Wildlife Department (TPWD) and Jeri's Seafood Inc., six public reefs and three private leases in Galveston Bay have been sampled monthly since December 1998. For the past year TPWD has provided monthly samples from San Antonio and Matagorda Bays. New Texas and Louisiana sites are being added. The website displays the most recent data from each site on the homepage and archives all data, such that a historical record is maintained. A web-accessible "Dermo Calculator" provides anyone with data on T, S and i-WI the opportunity to calculate a t-crit and explore the dynamics of disease progression.

An obvious limitation of the approach is that single values of T and S, taken at the time the oysters are sampled, are used to represent environmental conditions for the entire month. We are testing the utility of a continuous monitoring station to better predict disease progression ([www.luncon.edu](http://www.luncon.edu)).

# Gulf Oyster Industry Initiative

## Do Polychaete Annelid Mud Blister in Oyster Shells Serve as Reservoirs for *Vibrio vulnificus* Infections of Humans?

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\*presenter

*Vibrio vulnificus* (V.v.), a halophilic bacterium that occurs naturally in Galveston Bay waters, has been found in high concentrations in suspended particulate matter. V.v. can cause primary septicemia in immunocompromised humans, leading to serious illness and death. Most foodborne transmissions to humans result from consumption of raw oysters. Infections are only known to result from consumption of oysters on the half shell and not from consumption of oysters removed from their shells and placed in containers, indicating that some shell-associated factor is involved in transmission. Mud blisters caused by spionid polychaete annelid worms commonly occur on the interior surface of an oyster's shell. The blisters contain moist sediments from the surrounding seawater, so it may be possible that they occasionally serve as reservoirs for populations of V.v. If so, the mud blister has the potential to contaminate the surrounding oyster meat and subsequently infect humans who consume it should the blister become ruptured during shucking. If the meat is not immediately separated from the shell, as occurs in the half shell trade, chances of the released bacteria infecting the meat would be greatly increased. If oysters are promptly removed from their shells and placed in containers, however, chances of infection to human consumers would be greatly reduced by dilution in the fluid medium and decreased time of direct contact with the meat. Determining if a relationship exists between V.v. and mud blisters would, therefore, be a positive first step in understanding the mechanism of human infection with V.v. following consumption of raw oysters on the half shell.

Since June 2002, oyster samples have been collected monthly from eight subtidal and three intertidal stations within the Galveston Bay system. Several of the largest fluid-filled blisters found in each sample were ruptured in the laboratory and the blister's contents were tested for V.v. using the ELISA procedure. V.v. was found in 69.4 percent of the 199 subtidal mud blisters and in 51.6 percent of the 223 intertidal blisters examined thus far. V.v. levels in subtidal blisters were highest in summer (311.9 MPN/g) decreasing to a minimum in winter (4.5 MPN/g). V.v. levels in intertidal blisters were highest during the fall (111.0 MPN/g) decreasing to a minimum in the spring (13.1 MPN/g). Additionally, in two inter-tidal samples where the ELISA procedure was performed on both oyster blisters and meat, V. v. levels of blisters surpassed those of meats during the winter months, indicating that mud blisters may serve as overwintering refuges for the bacteria. Overall, the results thus far clearly support a V.v.-mud blister relationship. Oysters collected during the 2003 summer months are still being processed, and results from those will be added to the final project report, completion of which is expected in the fall of 2003.

# **Analysis and Numerical Modeling of Shoreline Change on Galveston Island**

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Numerical modeling of shoreline change on Galveston Island was conducted in order to gain understanding of and to design remedial action for an erosional hot spot at the west end of the island's seawall. The GENESIS model was chosen after careful consideration of that model's assumptions and after analysis of site conditions.

Analysis of shoreline data from 1956, 1965, 1990 and 2001 indicated that the period from 1990 to 2001, which was free from severe storms, had little cross-shore transport. Since GENESIS assumes no cross-shore transport, this period was chosen to set up the GENESIS model.

The model was driven by available Wave Information Study (WIS) waves that had been screened on the basis of the waves ability to generate the "observed" longshore sediment transport (inferred from a sediment budget). In addition, the numerical precision of the model was also given close scrutiny.

The model accurately calculated the shoreline change between 1990 and 2001 and it accurately calculated the beach response to a million-cubic-yard beach nourishment project in the Galveston groin field in 1995. The model was employed to project shoreline change into the future (2001 – 2011) under various beach nourishment scenarios that might limit the erosion of the hot spot. The simulations into the future were made assuming no significant cross-shore transport (due to a lack of storms) and assuming a certain level of cross-shore transport based on historical observations.

## **Could Extending the Jetties at the Entrance to Galveston Bay Alter Suspended Sediments at the Beaches of Galveston?**

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The objective of this experiment is to determine if alteration of the ends of the jetties at the entrance to Galveston Harbor may reduce the suspended sediments at the beaches on Galveston Island. It is clear from aerial photographs and buoy records that the large sediment loads coming from the distributaries of the Mississippi River provide significant fine

sediments that are carried near the shelf waters towards the Texas shoreline. A numerical model has been used to explore the effectiveness of altering the ends of the jetties to determine if a shift in the current regime would lead to reduced suspended sediments in the nearshore waters of Galveston Island.

## **Continental Shelf Edge and Upper Continental Slope, Northwestern Gulf of Mexico: Multibeam Bathymetry**

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Multibeam bathymetric data were collected by NOAA's U. S. Coast Survey over a large portion of the Continental Slope of the northwestern Gulf of Mexico during the early 1990s. Coverage extended upslope to a minimum depth of 150 m. More recently, the U. S. Geological Survey conducted multibeam surveys of a number of individual shelf-edge banks. With Sea Grant support we reprocessed the NOAA and USGS bathymetry to facilitate mapping and geological studies. Resulting imagery provides a new and exciting look at physiographic features of the outer Continental Shelf and upper Continental Slope: 1) Thick deposits of clay or silty clay at the shelf edge drape over the uppermost Continental Slope like drifting snow on the lee side of a windswept mountain ridge. 2) Along much of the northwestern Gulf of Mexico upper Continental Slope, N-S ridges and valleys lead from the shelf edge down the slope, the valleys typically ending in a deep basin. The valleys are conduits for slumps and gravity-induced sediment flow. At the westernmost extremity of the upper Continental Slope in this region, N-S ridges and valleys give way to a more open sloping terrain interspersed with domes. 3) Faults ranging in displacement from 5 to 50 m form outward-facing scarps near the shelf edge, especially around the heads of valleys. Some of the fault scarps are spoon-shaped, a characteristic of slumps in clay. Larger fault scarps, up to 400 m in displacement, segment the valleys farther downslope. 4) In a valley off the Sabine/ Trinity shelf-edge delta, a submarine canyon extends at least 25 km downslope and reaches a maximum relief of about 75 m. Branch canyons extend away from the main canyon, forming a dendritic drainage pattern. Submarine canyons and channels also are present downslope of the Brazos/ Colorado and Rio Grande shelf-edge deltas. A large slump scar extends downslope from the Brazos/ Colorado shelf edge delta. 5) Domes and banks are a dominant feature of the shelf edge and upper Continental Slope, but a few domes occur farther downslope. These are typically equidimensional in shape, often circular, as is characteristic of features formed over salt diapirs. Relief etched into the tops of domes and banks includes radial fault scarps and radial drainage channels

as well as solution-basins or sinkholes. Perched basins of varying sizes are a characteristic of the tops of domes, ridges, and banks. 6) Moats have formed around the bases of domes and banks. Seismic sections show moats around shelf-edge banks that are formed by nondeposition, following uplift or reef-building, and erosional beveling, of the bank tops.

## RNA:DNA Ratios as an Index of Zooplankton Under Varying Environmental Conditions

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Copepods are the dominant zooplankton taxa and form a critical trophic link between phytoplankton and fish. RNA:DNA ratios have been shown to correlate to growth rate and/ or egg production and serve as an estimate of nutritional condition and secondary production for several fish species and a few copepod species. This assay is based on the assumption that DNA remains constant once somatic growth is complete. RNA is an estimate of protein synthesis, so the RNA:DNA ratio is an estimate of protein synthesis ability per cell. The limited work on zooplankton has shown that RNA:DNA changes in response to ontogenetic change, differences in food and temperature. Measurements of nucleic acids on small individual zooplankton, such as *Acartia tonsa*, have not been made until now. A new highly sensitive fluorescent dye in combination with an automated microplate reader, have allowed us to make reading of nucleic acids in individual adult female *A. tonsa*. RNA:DNA ratios and egg production of wild *A. tonsa* were significantly higher for fed copepods versus starved over a 48 hr period. In addition, wild *A. tonsa* fed *Peridinium foliaceum* had higher RNA:DNA ratios and egg production than copepods fed non-toxic *Karenia brevis* or starved. *A. tonsa* has also been cultured at three different temperatures and three different salinities to determine how these environmental factors may affect RNA:DNA ratios. Preliminary findings suggest there is a strong interaction between temperature and salinity, which will alter nucleic acid values. We also plan to culture *A. tonsa* fed algae contaminated with heavy metals (Cd, Cu and Hg) in an attempt to determine how RNA:DNA ratios may change in the presence of environmental pollutants. Finally, *A. tonsa* will be sampled seasonally to examine natural changes in nucleic acid values of wild populations exposed to variations in temperature and salinity. We hope to use our data from cultured copepods to determine what factors may be altering nucleic acid values in nature. The ultimate goal is to apply this technique to field collected organisms to use as a proxy of secondary production.



# Development of Improved Monitoring Capabilities for *Karenia brevis* Using Gyroxanthin-based Detection Methods

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We have developed a combination filtration and high performance liquid chromatography (HPLC) method for detection of the harmful algal bloom dinoflagellate *Karenia brevis* in coastal waters. Our approach is based on detection of the biomarker pigment gyroxanthin and will allow reliable determination of *K. brevis* at concentrations lower than the FDA mandated limit of 5 cells ml<sup>-1</sup>. The development of this method consisted of two tasks. First, we tested five different types of glass fiber filters to determine the optimal combination of acceptable retention and fast filtration times for large volumes of water. We found that filtration through GF/C (NPR=1.2 mm) and 934-AH (NPR=1.5 mm) filters gave > 98 percent recovery of cells with filtration times ~ 10-20 minutes in water with relatively high suspended sediment load (Galveston Bay water). Second, we developed a "barebones" HPLC analytical procedure to optimize for the separation and quantification of gyroxanthin in mixed algal samples with an analytical run time of < 15 minutes. This combined filtration and HPLC method is an alternative or supplement to traditional cell counting approaches and is suitable for use by agencies interested in long term monitoring programs.

## Biogeography of Fishes of the Gulf of Mexico: Biodiversity and Biogeographical Affinities

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A total of 1,454 fish species in 681 genera and 221 families are documented from the Gulf of Mexico. These totals represent about 64.3 percent of the species, 79.4 percent of the genera, and 92.5 percent of the families recorded from the central western Atlantic. A majority of the fishes in the Gulf of Mexico are wide ranging continental fishes, wide ranging insular fishes and deep-sea benthic and pelagic fishes. Secretive insular fishes are less well represented. Despite the relatively high diversity, only about 5 percent of the fish species are endemic to the Gulf of Mexico. The majority of the endemic species are limited

to either the eastern, northwestern or southern sub regions of the Gulf. Only nine endemic species are ubiquitous throughout the Gulf. Based on percent endemicity, the Gulf cannot be considered a cohesive biogeographical region or province. However, based on its high diversity and unique warm temperate and tropical components, the Gulf is a distinct biogeographical area.

## **Corals at the Flower Garden Banks: Monitors of Environmental Change**

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Global-scale climate fluctuations affect the marine environment of the Gulf of Mexico. To anticipate future climate change and develop sensible policies for managing marine resources, we must understand climate variability and its effect on marine life. Our understanding would be greatly enhanced if we had better information about past climate changes and their effects. Such information is preserved in the skeletons of long-lived corals at the reefs of the Flower Garden Banks. Therefore, the goal of our project is to construct long, high-resolution records of skeletal density bands and chemistry, and to determine histories of local environmental conditions and coral growth from them. The field component of the study includes obtaining several 1.5 - 2 meter cores of skeletal material from massive *Montastrea* and *Siderastrea* coral heads at the Flower Gardens. These will be split in the laboratory and annual growth rates will be determined based on X-radiographic analysis of density bands. Samples of the skeletal material will then be taken with spacing such that near-monthly resolution is possible. The oxygen isotopic and Mg/Ca compositions of the skeletal material will be measured by mass spectrometry and used to estimate past water temperatures. We will verify the coral-derived records with twentieth-century instrumental data, and then use these records to characterize climate variability during the past 2.5 to 4 centuries. We have developed a computer model to simulate coral growth and geochemistry to assist us in better understanding how growth rate and calcification affect the coral record. Importantly, Flower Gardens corals also monitor the dominant pattern of winter climate variability in the extratropical Northern Hemisphere, the Pacific/North American (PNA) pattern. Since measurements that are now used to characterize the PNA pattern do not exist prior to 1946, the record we obtain should yield an important history of the PNA pattern. Analysis of these results will contribute directly to our understanding of the temporal character of interannual and interdecadal variations of winter climate. The relationship between changes of coral growth and environmental conditions will indicate processes affecting the corals, and long coral records will provide an important baseline perspective on the overall health and vitality of reefs.

# Effects of the Currently Used Herbicide Atrazine On Environmental Performance of Larval Red Drum, *Sciaenops ocellatus*

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Red drum larvae (*Sciaenops ocellatus*) were exposed to environmentally realistic and sub-lethal doses of the herbicide Atrazine to evaluate sublethal but ecologically critical effects on larval growth, behavior, survival potential and respiration rates. Larvae were reared to settlement size (6mm total length) and given an acute exposure of Atrazine for four days at three concentrations: 0, 50 and 100  $\mu\text{g}/\text{ml}$  (control, low and high, respectively). Tests of 96-h survival confirmed that these naturally occurring concentrations were sublethal for red drum larvae. Spontaneous and antipredator behavior and growth of larvae was monitored 1 and 3 days after exposure. Measurements of spontaneous behavior included: rate of travel, active swimming speed, net-to-gross displacement ratio (NGDR) and activity. Antipredator behavior was measured in assays involving an artificial visual predatory stimulus and an actual predatory fish. Oxygen uptake rates were also measured to assess the metabolic cost of Atrazine exposure. Measurements of startle responses elicited by the artificial stimulus included: responsiveness, response latency, maximum and average speed of the response, as well as the time to maximum speed. In the predation assay, responsiveness to an attack, response effectiveness, prey error and capture success were measured. Atrazine exposure showed an effect on all the four spontaneous swimming variables. Treated larvae exhibited a significantly faster rate of travel and active swimming speed, a more convoluted swimming path (smaller NGDR), and hyperactivity 3 days after treatment. Responses to artificial and actual predators were not affected by Atrazine exposure. Respiration rates of treated larvae were significantly lower than those of control larvae. The hyperactivity and faster swimming of exposed larvae indicate that sublethal and naturally occurring levels of Atrazine result in an elevated rate of energy utilization in red drum larvae, which is likely to increase their risk of death by starvation.

# Development and Application of Hypervariable DNA Markers (Microsatellites) to Issues in Red Drum Stock Enhancement

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The project had four objectives —

1. To develop hypervariable genetic markers (microsatellites) for use in ‘family-printing’ of red drum used in the stock-enhancement program of Texas Parks and Wildlife (TPW).
2. To assess the genetic component to variation in performance and survival of hatchery-produced fingerling red drum in the wild.
3. To estimate narrow-sense heritability for three traits critical to culture and/or stock enhancement.
4. To test up to 50 microsatellites for Mendelian segregation and independent assortment.

*Objective 1:* A total of 68 microsatellite loci were isolated and characterized from a red drum genomic library. A subset of 18 microsatellites were incorporated into “multiplexes” and family-printing of 148 TPW broodfish (86 females and 62 males) is nearly completed.

*Objective 2:* Progeny from 13 spawns were equalized (approximately 15,000-18,000 from each) and released into Packerly Channel Park (Corpus Christi Bay); 125 individuals from each spawn and 500 individuals from the released group were saved for genotyping. A total of 310 early-juvenile red drum was recovered over a nine-day period following release. These individuals will be genotyped once genotyping of the fish from each spawn has been completed. The (45) TPW broodfish used in this experiment, and 102 red drum sampled from the ‘wild’ (to be used as a reference ‘wild’ population), have been genotyped at a total of 31 microsatellite loci.

*Objective 3:* In summer 2002, 300 individuals from each of the 13 spawns were caged for planned use in three laboratory-performance assays. Following transfer to College Station, MMS (marginal metabolic scope) was assayed via respirometry on four individuals from each of the 13 spawns. Variation in MMS was found to be highly associated with variation in fish size and fish growth rate, but no significant fraction of MMS variation could be attributed to spawn. Shortly after the 2002 MMS assays, fish used in these experiments developed the viral disease lymphocystis, which precluded the planned subsequent assays of growth rate and cold tolerance under “common garden” conditions. In summer 2003, an additional set of MMS assays was performed; results seem generally consistent with those observed in 2002, but the analysis is not complete. The 2003 assays of growth rate and cold tolerance will begin in September.

*Objective 4:* These experiments are in progress. To date, 500 offspring from four of the 13 spawns have been genotyped at four microsatellites, permitting unambiguous assignment of the parents that produced the spawn. Genotyping of samples from the remaining nine ponds is in progress.

# Progress in the Research and Development of the Texas Farm-raised Live Bait Shrimp Industry

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The Texas Agricultural Experiment Station Shrimp Mariculture Research Facility, Corpus Christi (TAES-SMRF) has spent the past six years assisting Lone Star Farm (LSF), Austin, Texas in the development of sustainable and bio-secure live bait shrimp production technologies for the Texas market. The objectives of these efforts were to determine the most suitable candidate for live bait production. Studies were conducted with three commercially important native species, which include *Farfantepenaeus aztecus* (Atlantic brown shrimp), *F. duorarum* (Atlantic pink shrimp) and *Litopenaeus setiferus* (Atlantic white shrimp). Of these three species, *F. aztecus* was found to be the best overall candidate for bait shrimp culture. However, a significant lack of knowledge in basic husbandry for this species had to be overcome first before a domestication program could be initiated. With no dependable source of viral-pathogen-free (VPF) postlarvae (PL) to work with, the first task undertaken by TAES/LSF was to isolate a VPF broodstock from wild populations. Once a dependable supply of VPF PL was established, research efforts were shifted toward evaluation of the performance of this species in bio-secure closed recirculating induced maturation and larval rearing systems and in limited discharge nursery and grow-out systems. These studies have provided a comprehensive knowledge of the husbandry techniques for this species. The information collected is essential for the development of a sustainable and environmentally friendly production of farm-raised live bait shrimp in Texas.

Continuing efforts by Texas state regulatory agencies to reduce trawling for wild bait shrimp in regional bay systems will result in a decreased supply of live and dead bait shrimp products in the near future. The continuum of increasing demand and inconsistent wild supply necessitate the production of farm-raised live bait-shrimp to meet market demands. Furthermore, the continuing trend of low profitability in the commercial food shrimp industry due to low shrimp prices is forcing many Texas shrimp farmers to consider alternative strategies to increase farm revenues. Market studies were performed by the TAES/LSF researchers on retail live bait shrimp outlets in 1997 and a follow up survey will be performed in Fall 2003. The 1997 data and recent verbal communications show a definite eight-month gap in supply that a farm-raised product can easily fill. Based on these results, a pilot project is currently underway by the TAES/LSF team and another commercial shrimp producer to culture live bait shrimp as a supplemental crop. The 2004 production season will see several trials on farms to gauge the live bait shrimp market potential and assess the revenue generating capacity of live or dead bait produced on commercial shrimp farms.

# Origins of Red Drum Stocks: Assessing the Contribution of Different Nursery Grounds Using Chemical Signatures

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The aim of this study is to use natural markers in otoliths (trace elements and stable isotopes) to determine the nursery origin of red drum (*Sciaenops ocellatus*). In 2001 and 2002, age-0 red drum were sampled from nurseries in Texas: Sabine Pass, East Bay-Galveston, West Bay-Galveston, Matagorda Bay, Copano Bay, Aransas Bay and the lower Laguna Madre. Stable isotopes ( $d^{13}C$ ,  $d^{18}O$ ) and trace elements (Mg, Ca, Mn, Cu, Sr and Ba) were quantified in the otoliths of selected individuals from each nursery. Stable isotopes ratios differed significantly among estuaries and within site variability was relatively low. North to south gradients were observed for  $d^{13}C$  and  $d^{18}O$ , and both isotopes were enriched in otoliths of red drum from southern sites relative to northern sites. Correct assignment to a specific estuary based on stable isotopes ratios alone (i.e. cross-validated classification success from discriminant model) was greater than 80 percent in each year. Classification success was further improved when trace elements (e.g. Sr) were added to the model. Interannual variability in otolith chemistry was present; however, classification success was still high (78 to 88 percent) when year classes were pooled for four primary regions (Sabine to East Bay Galveston, West Bay Galveston to Matagorda, Aransas to Copano and Port Mansfield to Port Isabel), suggesting that signatures in the otoliths of red drum are useful indicators of nursery origin. We are currently in the process of predicting the nursery origin of sub-adult and adult (age-3 to age-5+) being collected in 2002 and 2003. To establish the nursery origin of sub-adult or adult red drum, stable isotope and trace element values in the core of the otolith must be determined. We have recently evaluated and developed drilling-based procedures to isolate otolith cores of red drum, and analysis of these cores is currently underway to predict the contribution of different nursery grounds to adult red drum stocks in Texas.

# Role of Natural Organic matter in Governing the Bioavailability of Toxic Metals to Estuarine Bivalves

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Estuarine bivalves, which are filter feeders, are capable of concentrating metals and organic pollutants from ambient water environments. Therefore, bivalves, especially oysters and mussels, have been extensively used as model organisms in environmental studies and as bio-indicator organisms in environmental assessment and monitoring programs by federal agencies (e.g., NOAA's NS&T program) to assess the bioavailable contaminant concentrations in coastal environments. Although metal bioavailability to aquatic organisms has received extensive attention over the last decade, metal uptake pathways and mechanisms need to be better understood to more effectively use bivalves as pollution indicators.

Since trace metals are often associated with potentially nutritious dissolved organic matter (DOM), absorption of DOM compounds by estuarine bivalves may control the uptake of trace metals and organic pollutants in a fundamental way. In our experimental work, we tested the hypothesis that metal's bioavailability, and thus the bioaccumulation in organisms, are regulated by the presence of specific DOM compounds, e.g., extracellular acid polysaccharides (APS), which are important components of colloidal macromolecular organic matter. Recent findings in our laboratory have shown that high molecular weight (HMW) DOM - as small as 0.04  $\mu\text{m}$  in size - and trace metals associated with it can be bioavailable to estuarine bivalves and that uptake of certain heavy metals (e.g., Cd, Cr, and Zn) by American oysters can be greatly enhanced in the presence of DOM in the experimental system. Our results also showed that oysters take up some  $^{14}\text{C}$ -labeled acid polysaccharide (APS) compounds, i.e., alginic acids or carrageenan, to a higher degree than the bulk of HMW DOM. This may be due to fibrillar acid polysaccharide molecules adsorbing on the gills, thus changing their surface charge, and modifying the concentration of gill adsorbing metals and their bioavailability to bivalves. Results of the short-term experiments with metals bound to HMW DOM also demonstrated that oysters could effectively filter metals bound to different HMW DOM fractions, with metals bound to specific APS compounds being removed from the water and incorporated into the oyster meat to a similar extent as natural HMW DOM. Thus, the quality aspect of natural DOM is as important factor for controlling bioavailability, removal and incorporation rates of metals into oyster meat as is that of quantity.

# Identifying Young Commercial Penaeid Shrimp Using Developmental and Molecular Approaches with Information on the Validity of the Genera *Farfantepenaeus* and *Litopenaeus*

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Based on morphology, the separation of adult brown shrimp (*Farfantepenaeus aztecus*) from pink shrimp (*F. duorarum*) is relatively easy. However, the separation of their postlarval and early juvenile stages is problematic. Characters for separating specimens between 10-25 mm TL are unreliable and depend on subjective interpretation of third pereopod (i.e., leg) length relative to the eye, rostral shape, uropod coloration, and/or early changes in the development of the genitalia. Difficulties in separating young brown from pink shrimp are compounded by intraspecific variability in morphology imposed by spatial, temporal, and environmental differences. The current descriptive literature has limited value for separating taxa because most studies do not consider the high degree of intraspecific morphological variability. Our approach for identifying, assessing, and verifying differences between species is to combine ontogenetic scaling techniques with specimen identification (ID) by molecular genetic testing.

In a previous meeting we reported the unambiguous molecular identification of shrimp by sequencing approximately 550 bp of the mitochondrial cytochrome oxidase I (COI) gene. Sequences for representative samples of pink and brown shrimp were characterized, and aligned against published sequences of the majority of *Farfantepenaeus* species. On the basis of this alignment, we developed a restriction assay using the endonucleases **Apo I** and **Mfe I** to discriminate among samples of brown, pink, and white shrimp (*F. setiferus*). However, this assay could not necessarily differentiate among exotic species of white shrimp (e.g., *F. vannamei*) that could also be present in Texas. Accordingly, we designed a single locus Multiplex-PCR assay that permits the discrimination of all members of *Litopenaeus* and *Farfantepenaeus* potentially found in the Gulf of Mexico.

The subdivision of the genus *Penaeus* into *Farfantepenaeus* and *Litopenaeus* based on morphological characteristics has been a subject of controversy. Molecular data based on COI (Baldwin et al. 1998) rejected the monophyletic origin of these two genera and indicated that *Litopenaeus* was paraphyletic with respect to *Farfantepenaeus* where *L. setiferus* emerged as sister taxa to *F. paulensis*. In contrast, the analysis of 16S rRNA mitochondrial gene (Maggioni et al. 2001) favored the monophyletic origin of these two genera. We characterized the mitochondrial DNA sequences for both COI and 16S rRNA for members of both genera and found that phylogenetic information contained in both genes supports the monophyly of both genera. Apparently, specimens of *F. aztecus* were misidentified as



*L. setiferus* by Baldwin et al. (1998), which lead these authors to erroneously reject the natural phyletic relationship of the members of *Litopenaeus*.

Our second **goal** is to develop an ontogenetic index for use by fishery biologists that permits a rapid and reliable separation of postlarval brown shrimp from pink shrimp. Accordingly, large numbers of juveniles and perhaps post-larval specimens could be ID without having to rely exclusively on molecular genetics techniques. For this purpose it is necessary to identify ontogenetic correlates on specimens whose identity has been validated with molecular techniques in a double blind study. The combination of genetic and developmental technologies should allow the unambiguous ID early stages (postlarval to juvenile) of brown and pink shrimp.