Toxic sulfide concentrations in the sediments and water column of the Suwannee River estuary and its influence on hard clam survival

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Project Number: PD-03-5

Project Duration: 5/27/2003-10/15/2003

Budget: $5000

Summary:

The hard clam *Mercenaria mercenaria* is an important aquaculture species that is grown to market size in estuarine sediments. Hydrogen sulfide, a natural metabolic poison known to decrease the survival and growth of many bivalve species, is often produced as a byproduct of organic matter decomposition in marine soft bottoms particularly in estuaries prone to periodic or sustained eutrophication. This study combined field surveys and laboratory experiments to determine whether sulfide is present in hard clam aquaculture areas and whether sulfide decreases the survivorship of two size classes of hard clams used in field aquaculture areas in the Suwannee River estuary. Sulfide was found in sediment porewater near and within high density lease areas (HDLA's) at concentrations averaging 0.079mM and as high as 0.567mM. The Derrick's Key, Gulf Jackson and Horseshoe Beach HDLA's were found to have significantly higher sediment porewater sulfide concentrations than the Pine Island and Pelican Reef HDLA's. Sulfide concentrations did not tend to vary predictably with sediment organic matter content, sediment grain size or most water quality parameters. Sulfide did vary predictably with salinity at some HDLA's. The survivorship of hard clam nursery seed (4-6mm) and grow-out seed (12-15mm) was reduced when exposed to sulfide in laboratory experiments. Addition of the antibiotic chloramphimicol tended to increase hard clam survivorship, suggesting that sulfide indirectly affects hard clam survivorship by facilitating bacterial proliferation. It was concluded that sulfide is present in the sediments of Florida's hard clam aquaculture areas at concentrations capable of reducing hard clam survivorship. However, with our current understanding, predicting which HDLA's and which lease areas within an HDLA are most at risk and when they are most at risk for potential toxic sulfide levels will require sampling of sediment porewater at specific planting locations and planting times.

Introduction:

The hard clam *Mercenaria mercenaria* is a common inhabitant of estuarine sediments and an important United States fishery species. In 2001, clam sales (162 million dollars) ranked seventh among US domestic fisheries; hard clam sales accounted for almost a third of this total value (National Marine Fisheries Service 2002). In Florida, hard clam (*Mercenaria mercenaria*) aquaculture has grown to become the third leading segment of the state's aquaculture industry, comprising over 18% of its $99 million total aquaculture sales in 2001 (USDA 2002). Currently, over 1,600 acres of state-owned, submerged land on the Atlantic and Gulf coasts of Florida are utilized as field nurseries for clam aquaculture, and much of this land is located within nutrient-rich (eutrophic) environments. Although proximity to sources of nutrient input helps to ensure an abundant food source, it also exposes hard clams to numerous potential stress factors, including the natural toxin hydrogen sulfide, which may decrease hard clam growth and survival.

In eutrophic environments, high nutrient inputs lead to increased rates of primary production followed by increased organic material availability (Borum and Sand-Jensen 1996). As this organic material is oxidized, the increased bacterial respiration rates can translate to reduced oxygen availability, particularly in benthic waters and sediments (Rosenberg and Loo 1988; Turner and Rabalais 1994). Sediments are especially prone to oxygen limitation because of reduced diffusion rates, and, as a result, deeper sediments are often completely anoxic. In environments lacking sufficient oxygen to support aerobic respiration, microbes utilize alternate electron acceptors (such as NO_3^- and SO_4^{2-}) and produce reduced compounds (such as N_2 and H_2S, respectively) as a by-product of organic matter decomposition (Diaz and Rosenberg 1995). Of these byproducts, hydrogen sulfide (H_2S) is particularly important from a biological perspective because it is a component of marine sediments worldwide (often reaching concentrations of several millimolar) and it is toxic to animal tissues in only micromolar concentrations (Fenchel and Riedl 1970, Bagarinao 1992). The primary toxic effect of sulfide is that it inhibits aerobic respiration in the mitochondria by binding to cytochrome c oxidase (National Research Council, 1979). Recent evidence also suggests that sulfide produces conditions favorable for bacterial proliferation, thus increasing rates of bacterial infection in sediment-dwelling animals (de Zwaan and Babarro, 2001).

The combined effects of hypoxia/anoxia and sulfide are known to reduce the growth and survival of many shellfish species, including various clams such as *Mulinia lateralis* (Shumway et al., 1983), *Macoma balthica* (Jahn and Theede, 1997), and *Donax serra* (Laudien et al., 2002). However, the direct impact of sediment sulfide on the commercially important hard clam *M. mercenaria* has never been examined. Because these clams feed and respire via siphons that extend just above the sediment surface, studies have typically explored the relationships among clam growth and various aspects of benthic water quality such as food availability and flow velocity (see Grizzle et al. 2001 for review). By contrast, few studies have tested the importance of conditions within the sediments on hard clam growth and survival. Some evidence suggests that hard clams grow slower in finer, muddy sediments than in coarser, sandy sediments (Pratt 1953). Pratt and Campbell (1956) suggested that one explanation for this depressed growth rate was the tendency of finer sediments to be less oxygenated and to contain more sulfide than coarser sediments. Furthermore, numerous studies have shown that

clams inhabiting vegetated habitats tend to grow slower than those inhabiting unvegetated habitats (Peterson and Beal 1989), although there is some evidence to the contrary (Peterson et al. 1984; Irlandi and Peterson 1991). The decreased flow rates, increased sedimentation rate and increased organic matter production within vegetated habitats all suggest a potential role for sulfide in hard clam survival and growth.

There is some anecdotal evidence to suggest anoxia and/or hydrogen sulfide may play a key role in clam survival at aquaculture sites on Florida's Gulf coast. High clam and oyster mortalities were observed to be associated with a layer of "black water" lying on the bottom at several sites currently being used as part of a field experiment of bivalve growth in the Suwannee estuary (Debra Murie, personal communication). The chemical characteristics of this water layer were not determined, but the investigators suspect it was at least anoxic. Several of the field personnel also reported a sulfur smell. Additionally, the shells of hard clams harvested from field nurseries often carry a dark gray or black stain characteristic of exposure to iron sulfides (personal observation). This suggests that hard clams in aquaculture areas are prone to sulfide exposure, but this has never been confirmed quantitatively. Because of the linkages between eutrophication and sulfide generation and the location of clam aquaculture areas in regions of potentially high organic matter input, understanding the potential role of toxic sulfide in decreasing harvests of the hard clam *M. mercenaria* is important to improving bottom culture practices.

This study integrated field surveys and laboratory experiments to determine 1) the sulfide exposure levels of hard clams and the relationships among sediment sulfide and various environmental parameters in the Suwannee River estuary, and 2) the effect of sulfide on the survival of two size classes of hard clams used in field aquaculture facilities.

Materials and Methods:

Field Sites

Water samples were collected weekly at 6 monitoring stations (one within the Suwannee River and one from near each of 5 High-Density Lease Areas (HDLA's): Gulf Jackson (GJ), Pelican Reef (PR), Derrick's Key (DK), Horseshoe Beach (HB) and Pine Island (PI) between May 27 and October 7, 2003 (Fig. 1). Monthly, water samples were collected from eight additional stations within the Suwannee River estuary but not in close proximity to HDLA's during this same time (Fig. 1). Sediment samples could not be collected at one monitoring station collected weekly (Off1) and at two monitoring stations sampled monthly (Off1.5, Off2) due to the depth of the water at these locations. Additionally, at the five HDLA's listed above, water samples were collected from within the sediments around living clams placed at these sites as part of a USDA-funded study of clam growth (Phlips et al, 1999) (hereafter referred to as "HDLA sites"). Between one and three individual lease sites were sampled at each HDLA. Water sampling within the HDLA's was performed twice: late-June/early-July 2003, and mid-September 2003.

Field Sampling

To quantify sulfide at the monitoring stations, water samples were collected from two positions in the water column and from sediemnt porewater. Using a van Dorn bottle, water was collected from 0.5m below the water's surface ("surface") and 0.5m

above the sediment surface ("bottom") from which 0.4 ml subsamples were taken using a 1.0 ml syringe. Two sediment samples were collected using a remote push core device similar to that described in Raz-Guzman and Grizzle (2001). From each push core, porewater samples were extracted from 5cm below the sediment surface by inserting a 1.0 ml syringe through sampling ports in the side of the push core. Sediment samples could not be collected from the monitoring station within the Suwannee River due to the depth of the water (>3m), so only bottom and surface water samples were collected at this station. For samples collected within HDLA sites, SCUBA was used to collect six water samples from 5cm below the sediment surface within the grow-out bags using 3.0 ml syringes. Additionally, within HDLA sites, three push cores were collected around the periphery of the grow-out bags and water samples extracted as described above.

All sediment porewater samples were immediately placed in a battery-operated centrifuge to rapidly remove suspended sediment, and two water samples (0.1 and 0.4ml) taken from the supernatant. Each individual water sample was placed into a 2ml microcentrifuge tube containing 1.0ml of a zinc acetate fixative solution (4:1 0.12M Zinc Acetate:1.5M NaOH) to prevent the auto-oxidation of sulfide. All samples were individually labeled and kept on ice until returned to the laboratory.

Following porewater extraction, the surface-most 10cm of sediment remaining in the push core was retained for later determination of sediment grain characteristics and total organic carbon. Water quality parameters (temperature, salinity, dissolved oxygen and pH) were determined 0.5m beneath the water surface and 0.5m above the bottom at each monitoring station using a Hydrolab Quanta (Hydrolab Inc.).

Laboratory Procedures

Once in the laboratory, sulfide concentrations were determined in water samples by spectrophotometry using the methylene-blue method of Gilboa-Garber (1971). Briefly, solutions of ferric chloride ($FeCl_3$) (0.2ml) and N-N-dimethyl-p-phenylenediamine (0.2ml) were added to each water sample. The blue-colored product (methylene blue) was read on a spectrophotometer (670nm) following a 15 minute incubation period. The absorbance values were then converted to concentrations based upon a standard curve using stock solutions of known sulfide concentrations. Those microcentrifuge tubes into which the 0.1 ml water samples were placed also had 0.3 ml of distilled water, effectively diluting the samples to ¼. This was necessary to quantify samples with absorbances above 1.0 where the absorbance-concentration relationship became curvilinear.

The sediments from each core were homogenized and equally divided (Half 1 and Half 2). Half 1 was weighed wet, dried to a constant weight, combusted in a muffle furnace (450^{o}C) and then weighed. Organic matter content was calculated as the change in mass following combustion. The sediment in Half 2 was analyzed for grain-size distribution following methods as described in Erftemeijer and Koch (2001). The sediment was weighed wet and flushed through a 0.063mm sieve; the retained fraction was then dried (60^{o}C) to a constant weight. The dried sediment was shaken through a sieve series and categorized as gravel (>2.0 mm), very coarse sand (1.0-2.0 mm), coarse sand (0.5-1.0 mm), medium sand (0.25-0.5 mm), fine sand (0.125-0.25 mm), very fine sand (0.063-0.125 mm) and silt/clay (<0.063 mm). Each fraction (excluding silt/clay) was weighed individually and its proportion calculated based on the total weight of dry

sediment. The fraction of silt/clay in Half 2 was calculated as the difference between the known dry mass of the sample prior to wet sieving (calculated from the wet weight:dry weight ratio determined for Half 1) and the combined masses of the gravel and sand fractions.

Statistical Analyses

Two-way ANOVA was used to determine whether interstitial porewater sulfide concentrations differed between different sampling times (Time) and between monitoring stations (Station). The sulfide concentrations and TOM of each monitoring station were compared using individual F-tests. Chi-square tests were used to determine whether sediment grain size distribution was independent of monitoring station and to compare each monitoring station. For each monitoring station, backwards stepwise regression was used to determine whether sulfide concentrations varied with various bottom (0.5 m from the sediment) water quality parameters and which parameters most reliably predicted sediment sulfide concentrations. To perform the backwards stepwise regression, the parameters temperature, salinity, dissolved oxygen concentration, pH, water depth and secchi depth were all entered into a multiple regression model. Parameters with the highest P-values were removed from the model one at a time as long as the F-value for the entire model was below 4.0. The removal of parameters ceased when the F-value rose above 4.0 or after all parameters had been removed.

Originally, for samples collected in and around clam grow-out bags, a fully nested sampling design was planned in which two lease sites within each of five HDLA's would be sampled at each of two sampling times (June/July and September). However, the often turbid water prevented consistent location of the mesh grow-out bags within and around which sampling was to occur. This resulted in a design only being applicable to two HDLA's, HB and PR. A two-way nested ANOVA was performed using sampling time (Time), HDLA (HDLA) and lease site nested within an HDLA (Lease) as factors where Lease was a random factor. Additionally, data from different lease sites within each HDLA were pooled and a full two-way ANOVA was performed to determine whether sulfide concentrations were significantly different between HDLA's and between the two sampling periods overall. Gulf Jackson was excluded from this analysis as bags were only located once at this HDLA.

Hard Clam Survival Experiments

The effects of hypoxia and sulfide on the survival of two size classes of clams (4-6mm nursery seed, 12-15mm grow out size) were examined. Survival of each size class was determined under the following conditions: 1) normoxia (100% of air saturation) without sulfide, 2) hypoxia (50% of air saturation) without sulfide and 3) normoxia with 0.350mM sulfide. Additional incubations were performed with the antibiotic chloramphenicol present or absent. The chloramphenicol treatment was necessary to separate the direct effects of sulfide from the indirect effects of colonization by bacteria on clam survival (de Zwaan and Babarro, 2001).

Clams were exposed to treatment conditions in a temperature-controlled, flow-through incubation system. Twenty-four nursery seed or eight grow-out seed were placed in each of five parallel chambers (constructed from clear polycarbonate tubing). Filtered seawater was drawn into five different equilibration chambers using a peristaltic pump.

In equilibration chambers 1 and 2, normoxic conditions were maintained by aerating the seawater with atmospheric air. In equilibration chambers 3 and 4, hypoxic conditions were maintained by constantly bubbling the seawater with nitrogen gas. In chamber 5, the seawater was brought to the desired oxygen and sulfide concentration using a precise mixture of N_2, O_2, and H_2S gases, as regulated by a three-channel digital mass flow controller (Cameron Instruments). The seawater was drawn from each equilibration chamber through adjoining incubation chambers containing the live animals via a peristaltic pump. For tests involving antibiotic effect, chloramphenicol was added to the filtered seawater prior to gas equilibration. Mortality was assayed as a failure of individual clams to close when they were removed from the incubation chambers and examined on a plastic tray.

Results

Sulfide Concentrations Near and Within High Density Lease Areas

Interstitial porewater samples contained sulfide at concentrations up to 0.567mM. At monitoring stations near the HDLA's, the overall mean sulfide concentration was 0.079mM (S.E. =0.009), and within individual monitoring stations, mean sulfide concentrations were between 0.021mM and 0.126mM (Fig. 2A). Sulfide concentrations varied significantly between sites and sampling times at the monitoring stations near HDLA's (Two-way ANOVA: Time: $F_{13,122} = 3.10$, $P = 0.001$; Station: $F_{4,122} = 6.58$, $P < 0.001$). Pair-wise comparisons showed the five monitoring stations falling into two groups: 1) DK, GJ and HB and 2) PI and PR. The stations within a group were not significantly different, but all between group comparisons were significantly different ($p<0.05$) (Fig. 2A). After correction for multiple comparisons using the sequential Bonferroni adjustment, PR was found to be significantly different than GJ and HB, but all other comparisons were not significant. Sulfide concentrations overall remained rather constant between May and July, but they increased substantially in August and returned to lower levels by late September (Fig. 3). At monitoring station not in close proximity to HDLA's (those sampled monthly), mean sulfide concentrations were between 0.064mM and 0.307mM (Fig. 2B). Sulfide was never detected at concentrations above the detection limit of the assay (0.0025mM) in bottom water or surface water samples within the water column.

Within hard clam grow-out bags, sediment porewater sulfide concentrations differed between leases within HB and PR (Two-way nested ANOVA: Time: $F_{1,47}=0.05$, $p=0.82$; HDLA: $F_{1,47}=0.85$, $p=0.454$; Lease: $F_{2,47}=4.71$, $p=0.014$). Around hard clam grow-out bags, sediment porewater concentrations differed between HB and PR (Two-way nested ANOVA: Time: $F_{1,18}=0.31$, $p=0.586$; HDLA: $F_{1,18}=25.1$, $p=0.034$; Lease: $F_{2,18}=0.11$, $p=0.898$). Overall, sulfide concentrations were not found to be significantly different between HDLA's or between sampling times within clam grow-out bags (Two-way ANOVA: HDLA: $F_{3,90}=2.15$, $p=0.100$, Time: $F_{1,36}=0.01$, $p=0.940$) or around the periphery of clam grow-out bags (Two-way ANOVA: HDLA: $F_{3,36}=1.38$, $p=0.265$, Time: $F_{1,90}=0.29$, $p=0.525$) (Fig. 4A,B).

Correlations Between Environmental Parameters and Sulfide Concentrations

Total organic matter was less than 2% at every monitoring station near the HDLA's (Fig. 5). HB possessed the highest TOM content (1.6%), while the other four

stations possessed similar TOM content (0.5-0.9%). The sediments were dominated overall by medium (0.25-0.5 mm) and fine (0.125-0.25 mm) sands, and the modal grain size at the individual monitoring stations was either medium (GJ and DK) or fine (HB, PI and PR) (Fig. 6). Sediment grain size distributions were not independent of monitoring station (χ^2 =482, df=24, p<0.001). In comparing each monitoring station, sediment grain size were significantly different at HB than at all other stations (Fig. 6). Sulfide was not significantly correlated with TOM or grain size between HDLA's or within individual HDLA's.

Using backwards stepwise regression, sulfide concentrations were found to increase with decreasing salinity both overall and at several of the individual monitoring stations (HB, DK and GJ) (Table 1). Sulfide also varied significantly with dissolved oxygen and secchi depth (at GJ and PI, respectively), but this relationship was not consistent across the different monitoring stations. The tendency of sulfide concentrations to vary with salinity coincided with those monitoring stations having higher sulfide concentrations (DK, GJ and HB; Fig. 2A).

Hard Clam Sulfide Tolerance

Survival of nursery, grow-out and larger seed was 100% under normoxic conditions even during the longest incubation period (25 days) (Fig. 7A-C). Survival of all size classes was lower under hypoxic conditions than under normoxic conditions (Fig. 7A-C). When sulfide was present, survivorship tended to be even lower than under hypoxia (Fig. A,B) although in larger seed clams (>15 mm) this was not apparent (Fig. 7C). However, when the antibiotic chloramphenicol was present, survivorship among grow-out seed exposed to sulfide was similar to that of grow-out seed under hypoxic conditions.

Sulfide concentrations did not remain constant over the course of the experiments apparently due to bacterial activity. Each experiment started with sulfide concentrations between 0.3 mM and 0.35 mM, but concentrations typically dropped to less than 0.1 mM by the end of each experiment. White bacterial blooms typically occurred in the sulfide treatment chambers within 3-4 days if no antibiotic was added and within 6 days if the antibiotic was added. Oxygen concentrations also tended to decline over the course of the experiments in the sulfide treatments chambers, typically approaching oxygen concentrations similar to that in the hypoxia treatment chambers by the end of the experimental period.

Due to high tolerance of *M. mercenaria* to hypoxia, experimental incubations had to be maintained for very long periods of time (up to 25 days). This reduced the number of replicates that could be performed up to this point (Table 2). These experiments will be continued until sufficient replication is achieved to allow meaningful statistical analyses.

Discussion

Hydrogen sulfide occurs in the sediments of Florida's west coast hard clam aquaculture areas at concentrations capable of reducing hard clam survivorship. Sulfide is known to be a common constituent of coastal sediments and to play a significant role in organizing communities of sediment dwelling animals (Diaz and Rosenberg 1995). This is the first study to demonstrate the presence of hydrogen sulfide in the sediments of hard clam aquaculture areas. The reduced survivorship of seed clams exposed to hydrogen

sulfide in the laboratory suggests sulfide could play an important role in hard clam mortality during the field aquaculture process.

Decreased survivorship in the presence of sulfide has been demonstrated in several coastal bivalves (for example: Shumway et al., 1983; Jahn and Theede, 1997; Laudien et al., 2002), but this is the first study to demonstrate such a response in the hard clam *M. mercenaria*. Both size classes of hard clams that are placed in lease areas, nursery and grow-out seed, showed reduced survivorship when exposed to sulfide in the laboratory. *M. mercenaria* lives beneath the sediment surface and extends its siphon into the water column, allowing it to constantly bathe its gills in oxygenated seawater. However, with its shell open, its mantle and foot tissues are directly exposed to pools of sediment porewater containing hydrogen sulfide. Many sediment infauna circulate oxygenated seawater through their body cavities or burrows, thus maintaining sulfide concentrations near their body tissues at lower levels than in the surrounding porewater. Such aeration is well-known in burrow dwelling invertebrates such as the lugworm *Arenicola marina* (Riisgard and Banta 1998), but the extent to which *M. mercenaria* aerates the sediment has not been studied. In this study, sulfide concentrations were determined in sediment porewater 5cm below the sediment surface. Hard clams near harvest size (~5cm) will be exposed to the sediment porewater at this depth, but the smaller seed sizes (0.4-1.5cm) used in the laboratory survival experiments will likely not. Additional data regarding sulfide concentrations at shallower sediment depths, adult hard clam sulfide tolerance and porewater aeration by *M. mercenaria* are needed.

Sulfide does not appear to cause a decrease in hard clam survivorship directly; rather it stimulates the proliferation of bacteria that, in turn, reduce hard clam survivorship. In the absence of antibiotics, visible bacterial blooms appeared within three days in the sulfide treatment incubation chamber. Over the course of the experiment, oxygen and sulfide concentrations in the sulfide chamber decreased, suggesting the proliferation of chemoautotrophic sulfide-oxidizing bacteria. When antibiotic was added, oxygen and sulfide decreased at a slower rate and hard clams survived longer than in the absence of antibiotics. Patterns of bacterial proliferation under sulfidic conditions have been shown to reduce the survivorship other coastal bivalves (de Zwaan and Babarro 2001), but the mechanism by which the bacteria do this currently not known. Considering that hard clams exposed to hypoxic treatments survived longer than those exposed to sulfide, it is unlikely that bacterially-mediated oxygen depletion was the cause of reduced survivorship in the sulfide treatment incubation chambers. More likely is that the bacteria foul the gills and labial palps of *M. mercenaria*, interfering with gas exchange and feeding processes.

Comparing the results of the survival experiments performed here to those of other investigators is complicated by differences in accompanying oxygen concentration in the sulfide treatments. Previous studies have only combined sulfide with hypoxic or anoxic conditions (Shumway et al., 1983; Jahn and Theede, 1997; de Zwaan and Babarro 2001; Laudien et al., 2002). To our knowledge, these are the first experiments in which sulfide exposure was combined with oxic conditions. Because hard clams circulate oxygenated seawater through their mantle cavity, the combination of oxygen and sulfide in the experiments provides conditions more realistic of those to which the clams are exposed *in situ*. The results presented here suggest that sulfide retains its toxicity (whether direct or indirect) even with oxygen present. Sulfide combined with hypoxic

conditions could reflect natural conditions HDLA's experience episodes of benthic hypoxia. Such conditions were not detected in this study, but we are testing the importance of combined sulfide and hypoxia on hard clam survivorship in ongoing experiments.

Sediment sulfide concentrations differed significantly among the five high-density lease areas sampled. Based just upon the average sulfide values obtained here, hard clams planted at the Derrick's Key, Gulf Jackson and Horseshoe Beach high-density lease areas face a greater risk for sulfide-related toxicity than those planted at Pine Island or Pelican Reef. However, predicting which HDLA's or lease sites are most at risk and when they are most at risk is not straightforward. When comparing the five HDLA's, sediment porewater sulfide concentrations were not correlated with sediment organic matter content, sediment grain size or water quality parameters. Among all the HDLA's, HB tended to stand out as the site with the highest sulfide concentrations, highest organic matter content and the finest sediments. A comparison of actual hard clam yields and grow-out times between HB and HDLA's with lower sulfide concentrations, lower organic matter content and coarser sediments (such as PI and PR) could clarify the potential importance of these sediment characteristics to the profitability of different HDLA's.

The data obtained by sampling sediment within and around clam grow-out bags emphasize the importance of small-scale variability in determining sulfide concentrations in sediment porewater of hard clam aquaculture areas. When comparing HB and PR, sediment porewater sulfide concentrations differed between the HDLA's and between different leases within the HDLA's. The pattern found in comparing the monitoring stations near the HDLA's (DK, GJ and HB with high sulfide and PI and PR with lower sulfide) was not strictly reflected in comparisons of samples taken within and around the grow-out bags. This indicates that, although the measurements taken at the monitoring stations provide a general guideline for which HDLA's are most at risk, to evaluate the actual potential sulfide exposure level of hard clams on particular lease sites within the HDLA's will require direct measurement at the lease site of intertest.

Mean sulfide concentrations peaked during late August and early September while remaining relatively low and constant during the remainder of the sampling period. This coincides with the annual temperature peak when episodes of benthic hypoxia are most prevalent in the northern hemisphere (Rosenberg 1980). However, benthic hypoxia was never detected during this period in the Suwannee River estuary and sediment porewater sulfide concentrations did not significantly correlate with temperature. At those high-density lease areas with the highest sulfide concentrations (DK, GJ and HB), sulfide concentrations tended to vary with salinity, but this relationship did not hold at those HDKA's with lower overall sulfide concentrations (PI and PR). One of the potentially complicating factors in this analysis was that the water quality parameters were collected at the same time as the sediment sulfide concentrations. Because exchanges between sediment porewater and the water column do not occur instantaneously (Graf 1992), there likely exists a time lag between changes in water quality parameters and associated changes in sediment porewater chemistry that would confound the relationships examined here. Real-time water quality data is coming available at three of the HDLA's monitored in this study (GJ, HB and PI). This data has been recorded at 30-minute intervals since winter/spring of 2002, and will allow us to

determine whether a lag time exists between sediment porewater sulfide concentrations and water quality parameters around HDLA's in the Suwannee River estuary.

Ongoing Research and Future Directions

Studies involving both the field survey data and survival experiments are ongoing. Sediment porewater sulfide concentrations will be correlated with real-time data being recorded by water-quality monitors at three HDLA's in the Suwannee River estuary. This will allow us to determine not only whether porewater sulfide concentrations can be predicted from water quality parameters but also what the time-lag is between changes in water column conditions and changes in sediment conditions. Simultaneously with the data in this study, phytoplankton productivity data were collected by other investigators. Collaboration with those investigators will allow us to determine whether changes in porewater sulfide concentration were associated with phytoplankton blooms. Survival experiments involving the hard clam *M. mercenaria* will continue not only to replicate those treatments already performed but also, if funds permit, to introduce additional treatments. In addition to the sulfide+normoxia treatment, sulfide+hypoxia would allow us to determine whether periods of low oxygen availability increase the susceptibility of hard clams to sulfide-related mortality.

The results of this study suggest several important directions for further investigation. Studies of hard clam survival under different sulfide levels in the field are needed to determine whether survival curves generated in the laboratory translate to actual losses in aquaculture areas. Additional measures of the stress sulfide places on hard clams, such as changes in growth and condition (sometimes referred to as "fatness") and expression of protein biomarkers, are also needed. Potentially, protein biomarkers offer a rapid method for assessing the short-term molecular response of hard clams to specific environmental stressors, and as such, they could be used as a basis for management decisions prior to the onset of terminal stress indicators such as high mortality. Further, the mechanism by which bacteria reduce hard clam survivorship is not known and deserves attention. While bacterial proliferation cannot be prevented under field conditions, its impact on aquacultured *M. mercenaria* could be minimized by understanding under what environmental conditions proliferation occurs. This would involve knowing not only what ambient environmental conditions facilitate bacterial growth, but also how the hard clam modifies its local sedimentary environment. The latter may include aeration of sediment porewater by individual hard clams as well as the collective effects of densely-stocked hard clams on local organic matter levels via deposition of feces and pseudofeces.

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Table 1. Results of backwards stepwise regression involving interstitial sulfide concentrations and water quality parameters (temperature, salinity, dissolved oxygen, pH, water depth, secchi depth). Shown are the parameters remaining in the model when the F-value rose above 4.0 and the coefficient and p-value of each parameter in the final regression model.

Station	Parameter	Coefficient	P-value
DK	Salinity	-0.068	0.032
GJ	Salinity	-0.103	0.021
	Dissolved Oxygen	-0.365	0.029
HB	Salinity	-0.072	0.033
PI	Secchi Depth	-0.412	0.055
PR	None Remaining	----	----
All Station Mean	Temperature	0.046	0.172
	Salinity	-0.054	0.017

Table 2. Replication of hard clam survival experiments. First number shows the number of experiments run for particular treatments, and the number in parentheses indicates total length of incubation period (determined either by death of all animals in hypoxia and sulfide treatments or by logistical constraints).

	- Chloramphenicol			***+ Chloramphenicol***		
	Nursery	**Grow-out**	**Larger**	**Nursery**	**Grow-out**	**Larger**
Normoxia	1 (25)	1 (25)	1 (8)	1 (13)	1 (13)	0
Hypoxia	1 (25)	1 (25)	1 (8)	1 (13)	1 (13)	0
Sulfide+Normoxia	1 (25)	1 (8)	1 (8)	0	1 (13)	0

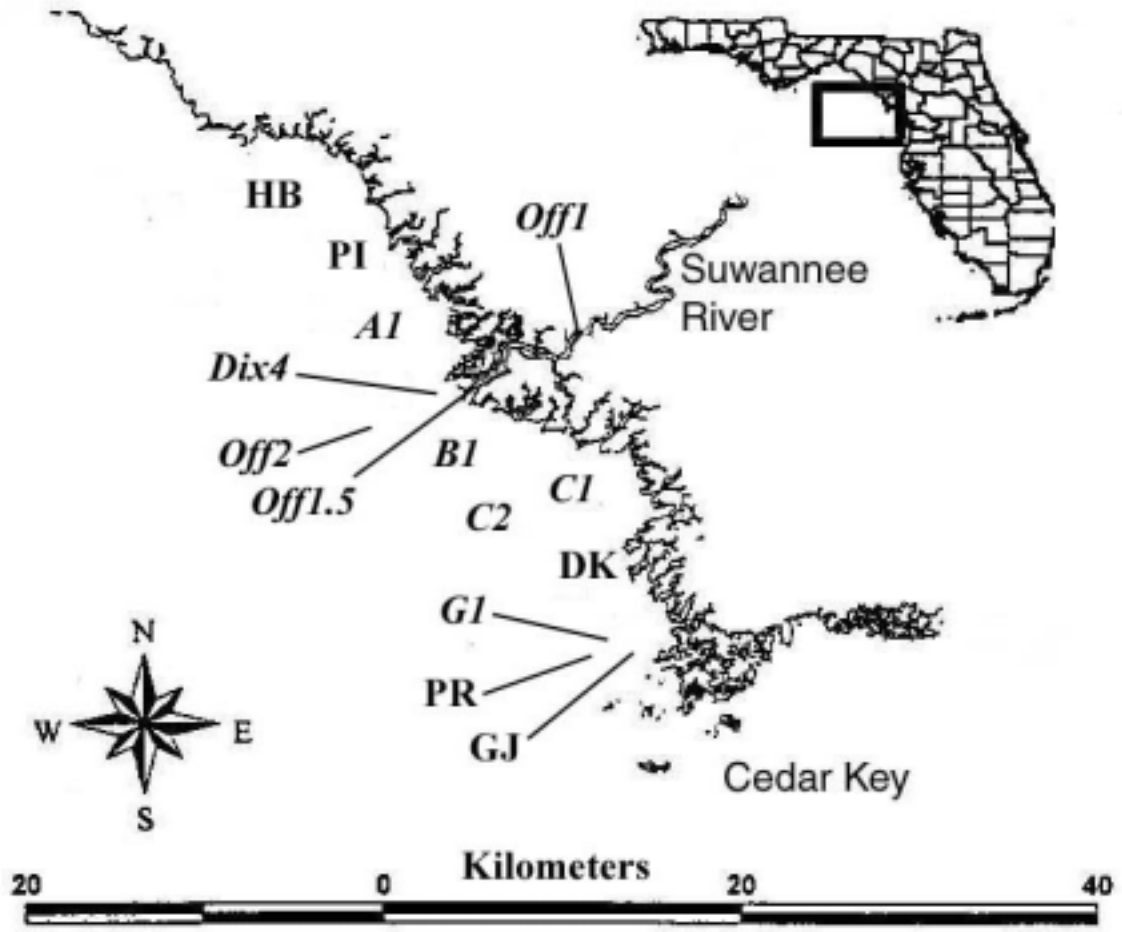

Figure 1: Sampling locations in the Suwannee River estuary. DK—Derrick's Key, GJ—Gulf Jackson, HB—Horseshoe Beach, PI—Pine Island, PR—Pelican Reef. Modified from Phlips et al. 1999. Other letter designations in italics indicate monitoring stations not in close proximity to high-density lease areas.

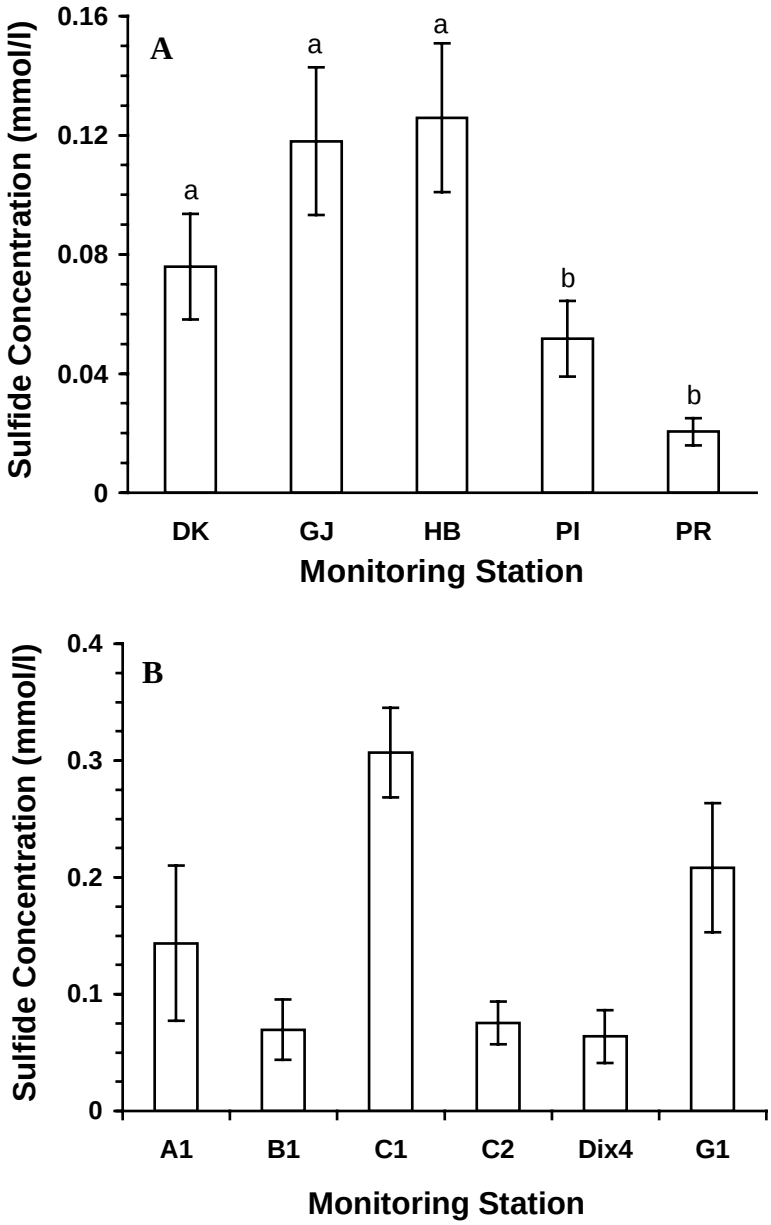

Figure 2: Mean sulfide concentrations in sediment porewater at A) each of the five monitoring stations near HDLA's and B) each of six monitoring stations not in close proximity to HDLA's. Different letters indicate monitoring stations that were found to be significantly different using individual F-tests ($p<0.05$). DK—Derrick's Key, GJ—Gulf Jackson, HB—Horseshoe Beach, PI—Pine Island, PR—Pelican Reef.

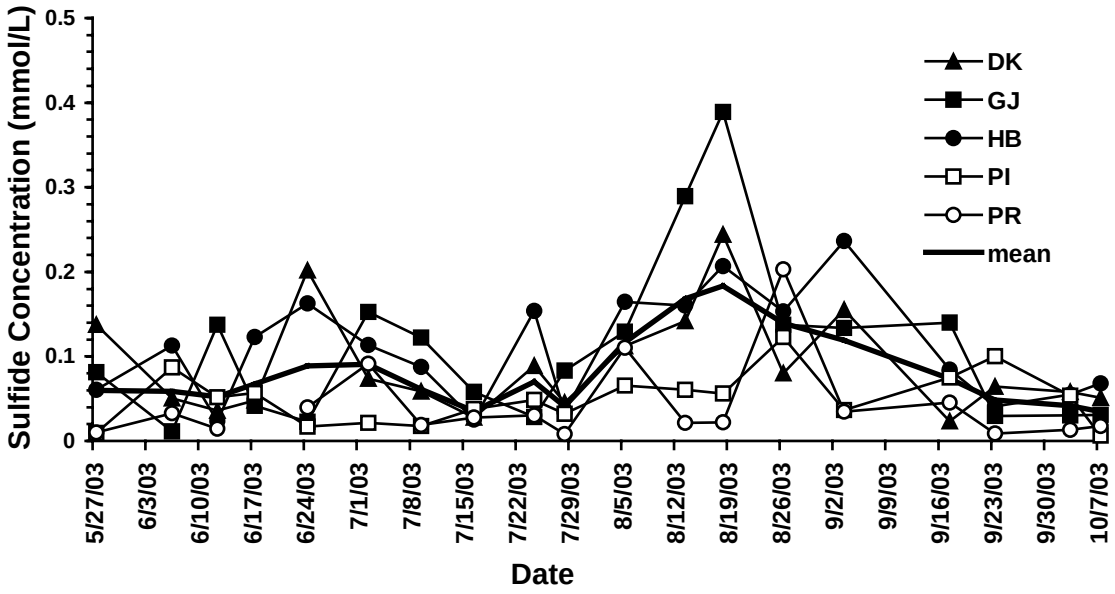

Figure 3. Mean porewater sulfide concentrations at each of the five monitoring stations near HDLA's between May 25 and October 7, 2003. Heavy line shows mean of all samples collected during each sampling period. DK—Derrick's Key, GJ—Gulf Jackson, HB—Horseshoe Beach, PI—Pine Island, PR—Pelican Reef, SK—Sandfly Key. Each point is mean of two samples.

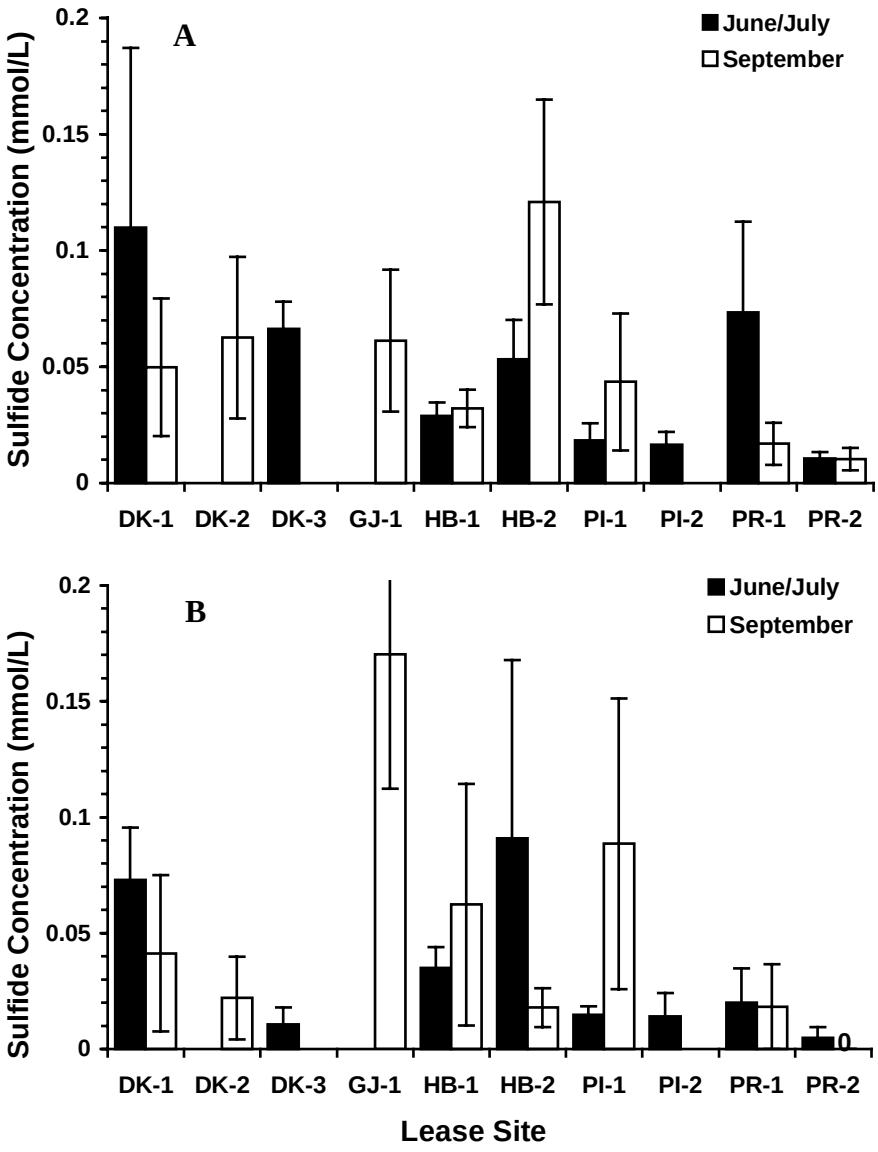

Figure 4. Mean sulfide concentrations in sediment porewater A) within grow out bags and B) at the periphery of grow out bags on different leases within the five HDLA's. DK—Derrick's Key, GJ—Gulf Jackson, HB—Horseshoe Beach, PI—Pine Island, PR—Pelican Reef, SK—Sandfly Key.

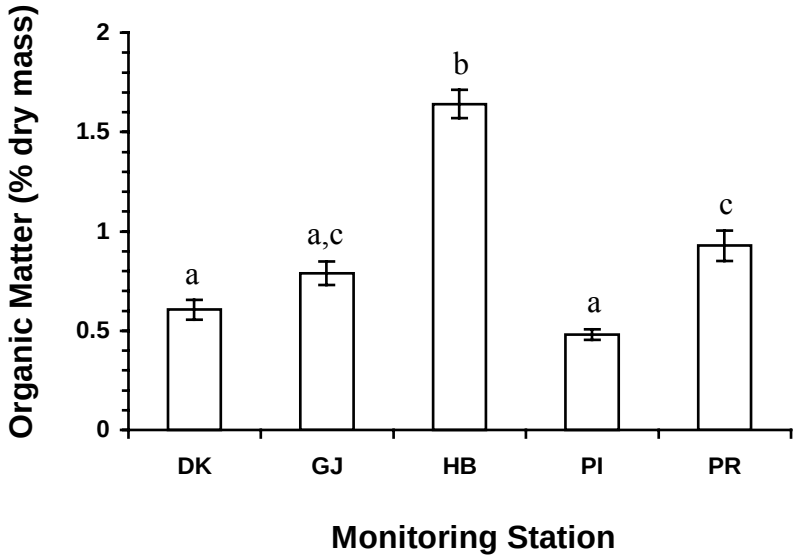

Figure 5. Mean sediment total organic matter at each of the five monitoring stations near HDLA's. Different letters indicate monitoring stations that were found to be significantly different using individual F-tests ($p<0.05$). DK—Derrick's Key, GJ—Gulf Jackson, HB—Horseshoe Beach, PI—Pine Island, PR—Pelican Reef.

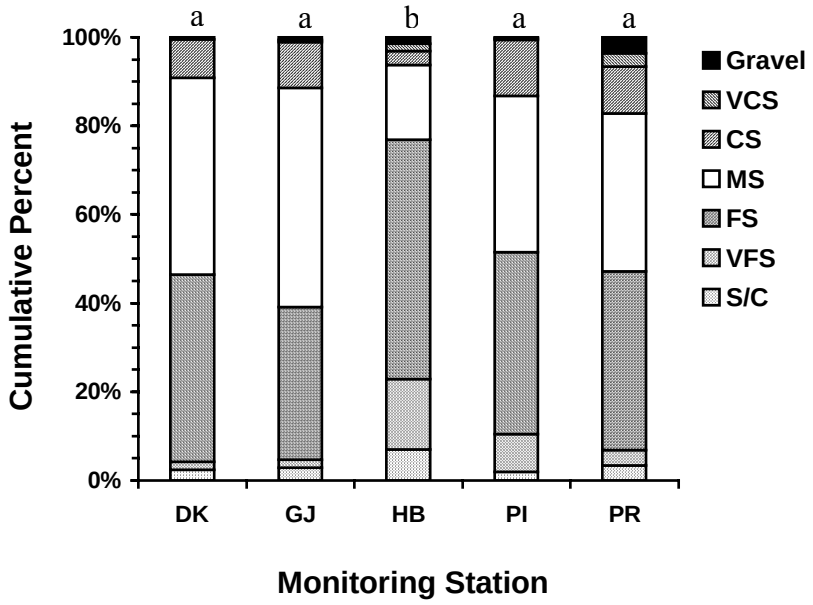

Figure 6. Mean sediment grain size distributions at each of the five monitoring stations near HDLA's. Different letters indicate monitoring stations that were found to be significantly different using chi-square tests ($p<0.05$). DK—Derrick's Key, GJ—Gulf Jackson, HB—Horseshoe Beach, PI—Pine Island, PR—Pelican Reef. Gravel: >2.0 mm, VCS—very coarse sand: 1.0-2.0 mm, CS—coarse sand: 0.5-1.0 mm, MS—medium sand: 0.25-0.5 mm, FS—fine sand: 0.125-0.25 mm, VFS—very fine sand: 0.063-0.125 mm, SC—silt and clay: <0.063 mm.

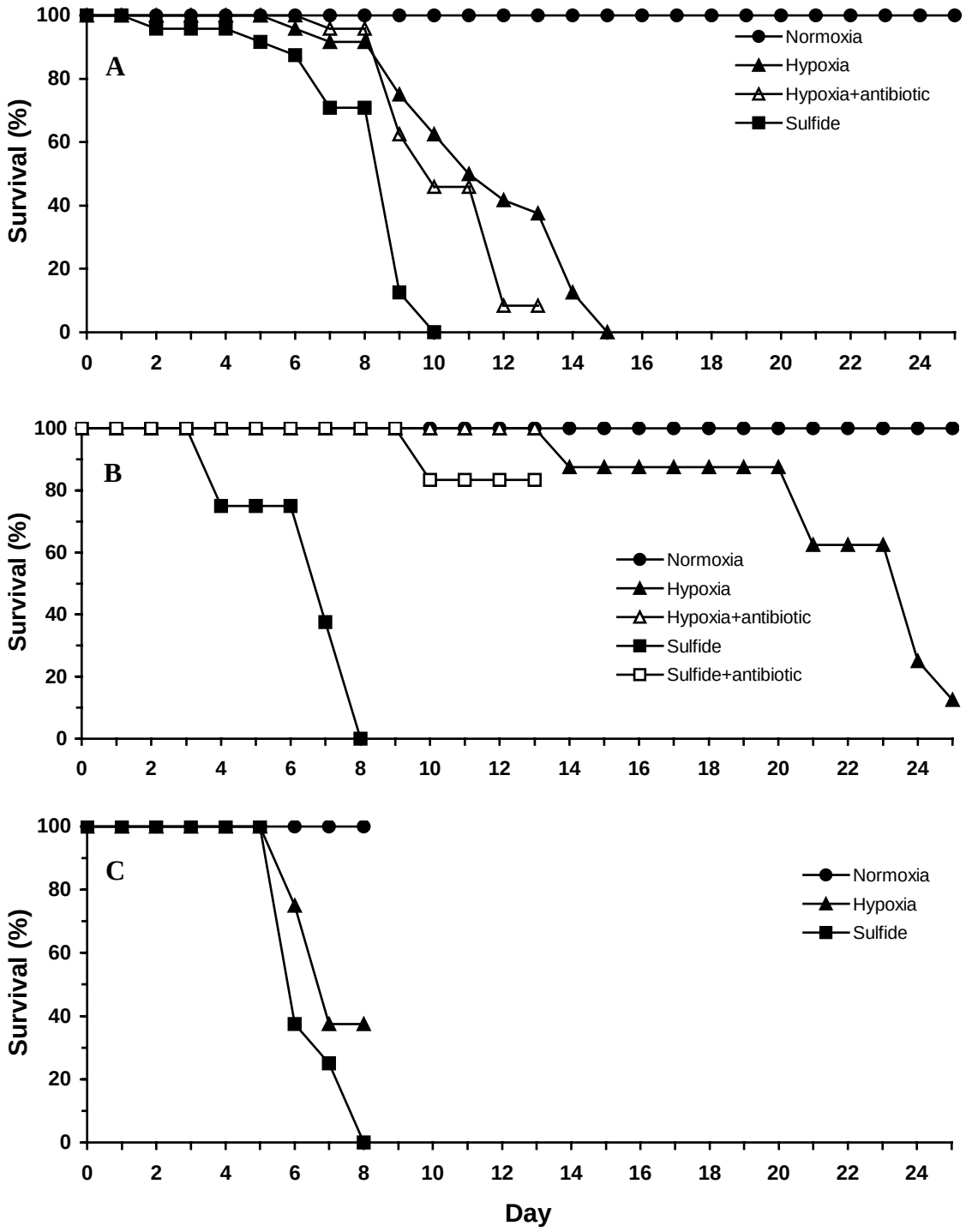

Figure 7. Percent survival of clams in each of three size classes A) nursery seed (4-6 mm), B) grow-out seed (12-15 mm), and C) larger seed (>15 mm) exposed to normoxia, hypoxia and sulfide.