

Biological effects of suspended sediments on shellfish in the Charlotte Harbor Watershed –
implications for water releases and dredging activities

Final Report

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Project Manager:
Ms. Catherine Corbett

by
Drs. Aswani K. Volety and Vincent G. Encomio

Florida Gulf Coast University
10501 FGCU Boulevard South
Fort Myers, Florida, 33965-6565
e-mail: avolety@fgcu.edu

The Charlotte Harbor National Estuary Program is a partnership of citizens, elected officials, resource managers and commercial and recreational resource users working to improve the water quality and ecological integrity of the greater Charlotte Harbor watershed. A cooperative decision-making process is used within the program to address diverse resource management concerns in the 4,400 square mile study area. Many of these partners also financially support the Program, which, in turn, affords the Program opportunities to fund projects such as this. The entities that have financially supported the program include the following:

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Introduction

The Caloosahatchee Estuary has been significantly altered both by hydrologic modifications and by increased development of adjacent lands for agricultural, residential, and commercial use. These alterations have impacted water quality (e.g., salinity, turbidity, nutrient loads) and have resulted in the loss of oyster reefs and other bivalves, thus disrupting the nursery habitat of crustaceans and fishes within the estuary. Many anthropogenic activities in or near aquatic habitats re-suspend bottom sediments and create turbid conditions that differ in scope, timing, duration and intensity from re-suspension events induced by storms, freshets, or dredging (Wilber and Clarke 2001). The Caloosahatchee Estuary receives periodic freshwater releases from Lake Okeechobee. These releases are regulated by the opening and closing of weirs upstream, where lands are subject to agricultural practices, and to pesticide and heavy metal usage. Pesticides and heavy metals are bound to the organic matter around sediment particles and are carried downstream with massive freshwater releases. Due to their filter feeding nature, benthic bivalves such as clams and oysters are exposed to high sediment concentrations and/or contaminant loads associated with the sediments. In addition, heavy metals and organic pollutants originating from marina operations and boat docks are released into the water, are bound to sediments, and are then deposited on the bottom where they are unavailable to filter feeding organisms. These sediments that are deposited and buried along the estuary can be re-suspended as a result of periodic freshwater releases, and dredging activities, thus making them bio-available once again.

In order to improve these conditions it is critical to understand the connections that exist between land use and impacts on estuarine ecosystems. Such information is clearly necessary, but currently lacking. Suspended sediment can have numerous ill effects on estuarine life (see Wilber and Clarke 2001). While some studies have examined the impacts on benthic communities as a consequence of dredging (Kaplan et al., 1973, Van Dolah et al., 1984, and Clarke et al., 1993), effects of re-suspended sediments on fish and shellfish are not well examined. Suspended sediments affect the physical, chemical and biological aspects of aquatic environments. For example, increased light attenuation due to turbidity reduces visibility, thereby affecting sea grasses. Sediment re-suspension due to periodic releases of freshwater and dredging activities increases turbidity and enhances contaminant loads in the water, thus increasing bio-availability of these contaminants to aquatic organisms. Increased sediment concentration in water may also result in egg abrasion, reduced bivalve pumping rates, and direct mortality (see Wilber and Clarke 2001).

Mobile organisms such as fish can move away from areas of increased sediment loads, but filter feeding benthic organisms such as oysters and clams are at particular risk due to their sessile nature. Suspension feeding animals, as primary consumers of organic carbon, feed on biological particles (detritus and phytoplankton) that are roughly the same size of suspended silts and clays. These sedimentary particles can foul an organisms' filtering apparatus and require the investment of high levels of metabolic energy for removal, energy that would otherwise have been used for physiological activities such as growth and reproduction. This can lead to death by smothering or hypoxia/anoxia, to reduced fitness due to lower reproductive energy allocation, or to poor health due to immunologic stress. Oysters and other bivalves respond similarly when influenced by environmental stressors (e.g., organic contaminants, see Chu and Hale 1994, Chu 1999, Chu et al. 2002, Capuzzo 1996; and sediment-contaminant mixture, see Cruz-Rodriguez et al., 2000). Due to their lipophilic nature, pesticides and PCBs are bound to organic matter around sediment particles.

This association is particularly harmful for suspension feeders due to their dependence on filtration for food and gas exchange. In addition, the ability of bivalves to breakdown organic pollutants is limited.

Many of the contentious existing as well as pending land- and water-use practices within the Charlotte Harbor watershed affect the suspended sediment load within the watershed. As stated earlier, freshwater releases, maintenance dredging, and construction of new homes and docks along the coast will result in increased sediment loads. The pulsed release of freshwater through water-control structures upstream in the Caloosahatchee River has an impact many miles downstream within the brackish waters of the estuary. Abrupt changes in salinity cause clay flocculation and higher sedimentation rates. In addition, the greater occurrence of urban, agricultural, and industrial land use results in higher levels of organic pollutants transported by suspended sedimentary particles entering the Caloosahatchee River.

With support from the South Florida Water Management District (SFWMD) and the Charlotte Harbor National Estuary Program (CHNEP), we have initiated studies that investigate the effects of watershed alteration (specifically salinity), and pesticide and heavy metal accumulation in oysters in the Caloosahatchee River and estuary. Specifically, we have been investigating responses of oysters (e.g., oyster growth, spat recruitment, disease incidence, reproductive potential and condition index) to salinity and contaminant stress, and we have been examining the role that oyster reefs play as essential fish habitat. Through our studies we have identified the effects of prolonged freshwater discharges (or lack thereof) as well as and contaminant loads in oysters at various locations of the Caloosahatchee Estuary. We have identified areas that currently do not have established oyster reefs, but that have the potential for the development of successful oyster reefs under current water-management practices / salinity regimes. These areas support rapid growth of juvenile oysters, attract abundant oyster spat, have reproductively active oysters, and exhibit high oyster condition index—essential ingredients for the development of healthy reefs. Our results suggest that healthy oysters attract greater numbers and possess a greater diversity of estuarine organisms than do either dead oysters or bare bottom. Species collected in association with oyster reefs include decapod crustaceans (e.g., white shrimp, grass shrimp, snapping shrimp, porcelain crab, blue crab, common mud crab, black-fingered mud crab, stone crab) and fishes (e.g., Gulf toadfish, skilletfish, sheepshead, pinfish, Florida blenny, feather blenny, frillfin goby, code goby) underscoring the importance of bivalves in this ecosystem. In addition, oysters and clams provide critical ecological functions within the estuary through the filtration of the water column, the creation of a reef and benthic structure for habitat and/or refuge, and the provision of food for other estuarine organisms. In short, oysters and clams provide additional habitat structure, habitat diversity, and habitat integrity to the estuary. We have also identified beds of the clam *Rangia cuneata* within the upper Caloosahatchee River. Dense aggregations of this clam occur within these beds and this species may play an important role in the water quality and bottom-up control of phytoplankton and nutrients in low salinity regions of the estuary. The soft-bottom habitats that these clams occupy are also the estuarine areas where effects of water releases and re-suspension of sediments might first be observed. However, rates of sediment deposition, and the amounts of suspended sediments in these areas are not known.

In southwest Florida, anthropogenic alterations to estuaries such as the Caloosahatchee River now result in increased frequency and intensity of freshets. Increased downstream flow can result in increased sediment loads and re-suspension of sediment in shallow water areas. *Crassostrea*

virginica is the most prominent bivalve found in the mesohaline (10-30 ppt) regions of the Caloosahatchee estuary. The health of oysters and the habitats they create are directly influenced by anthropogenic activities such as freshwater releases and dredging. The clam *Rangia cuneata* is commonly found in soft-bottom estuarine habitats of the eastern and Gulf of Mexico coasts of the U.S. *Rangia cuneata* was chosen as a test organism because it is a dominant bivalve representative of oligohaline (<10 ppt) portions of the Caloosahatchee estuary and therefore a potentially effective bio-indicator of watershed alterations in this system. The objectives of the experiments were to test:

- i) the effect of sediment size
- ii) the threshold (maximum levels) levels of sediment loads
- iii) the effect of prolonged sediment exposure

This project addresses, directly and indirectly, all three priority problems: hydrologic alterations, water quality degradation, and fish and wildlife habitat loss. Hydrologic alterations and dredging operations increase sediment loads in the water, thus compromising water quality, due both to increased sediment loads, and to enhanced contaminant loads. Negative impacts on shellfish (oysters and clams) such as decreased energy reserves, lower condition index, and increased mortality, translate to poor habitat quality for the forage of commercially and recreationally important fish. Through our proposed project we will be able to identify threshold levels of sediment loads, areas of high sedimentation, sediment size, and duration of sediment exposure to bivalves that will negatively impact their physiological and ecological functions. These results will provide baseline data for development of water quality information that impairs organismal function as well as information pertaining to land use management in order to improve the conditions of the ecosystem.

Material and methods.

In situ measurements of sediment loads

Sediment traps were placed strategically throughout the Caloosahatchee River at 5 sites (Iona Cove, Cattle Dock, Bird Island, Kitchel Key, and Tarpon Bay; see Figure 1). These sites are locations where oyster reefs are monitored continuously for condition index, growth, reproduction and recruitment as part of a larger study examining the effects of freshwater releases on oyster reefs and their associated communities. Three traps made of PVC pipe (4 in. diameter/1 ft length), were deployed at each site for 30-day intervals, over a 3-month period. Sediments were brought back to the laboratory to be dried and weighed to determine total sediment loading rates of sedimentation at those sites.

Oyster Experiments

Oyster collection and maintenance

Oysters were collected from Bird Island in the Caloosahatchee Estuary. After transport back to the laboratory, oysters were acclimated for 10 days in aerated artificial seawater (Instant Ocean) at 25 parts per thousand (ppt). During the acclimation period and throughout the experiment oysters were fed daily at 0.1 g of commercial algal paste (Instant Algae) per individual oyster. After the acclimation period, oysters were placed in 10 gallon aquaria and acclimated to tank conditions for an additional 10 days. Water was changed and tanks were cleaned twice a week.

Laboratory sediment exposures

A total of three test doses were tested for two sediment sizes, silt (40-60 μM) and clay (< 4 μM). Aquaria were dosed with 0 (control), 1.5 and 2 gm of clay- or silt-sized sediment particles/oyster/day. Commercially available sediment (Ward Scientific) was used in this experiment. A total of 18 aquaria were used resulting in 3 replicate tanks per treatment (2 sediment types x 3 sediment doses/sediment x 3 replicate tanks/treatment). Ten oysters were placed in each tank. Oysters were exposed to sediment treatments for 5 weeks. This experiment was repeated three times and results are reported separately (Experiment I, II and III).

Oyster dry weight and condition index

Condition index of oysters (n = 10 oysters/replicate tank) from laboratory and field experiments were analyzed according to Lucas and Beninger (1985), which expresses condition index as the ratio of meat weight to shell weight. Oysters were shucked open, and the meat was separated and placed into pre-weighed plastic tubes. Soft tissues were freeze-dried for 48 hours while shells were dried in an oven at 65°C for 24 hours. The dry tissues and shells were then weighed and the condition index was calculated as dry meat weight/dry shell weight x 100.

Glycogen analysis

Glycogen content of homogenized freeze-dried oyster tissue was estimated according to Van Handel (1965) and Baturu et al. (1995), using the anthrone reagent method. After weighing total oyster tissue, the dry tissue was pulverized to yield a homogenized powder. A portion of this dry powder was weighed (~50 mg) and diluted in a phosphate buffer solution (50 mM sodium phosphate, 1mM EDTA and 0.5 mM phenyl methonyl sulfate, pH 7.2) and mechanically homogenized to produce a wet tissue homogenate. The homogenate was then digested in boiling potassium hydroxide (30% w/v) and glycogen was precipitated with 95% ethanol and saturated sodium sulfate. The glycogen precipitate was dried overnight at 60 °C. Anthrone reagent (0.15 % in 72% sulfuric acid) was added to the precipitate and incubated at 90 °C for 20 min and then cooled in ice. Positive reaction with the anthrone reagent produced a blue color, directly proportional to the amount of glycogen contained within the sample. Samples were added to a 96-well microplate and read on a microplate spectrophotometer at 620 nm. Commercially purified oyster glycogen (Sigma Biochemicals) was used to generate a standard curve for each set of microplates. Glycogen content was expressed as mg glycogen/g dry weight of tissue.

Statistical analyses

Lab experiment data (condition index, dry tissue weight and glycogen) was analyzed by analysis of variance (ANOVA). A mixed model (Model III) ANOVA was employed examining the effects of sediment type (clay vs. silt), sediment dose (0, 1.5 and 3.0 g) and their interaction (sediment type x sediment dose). Additionally, individual oysters sampled from each tank (Tank) were treated as a random nested factor within dose [tank(dose)] to avoid problems of pseudo-replication (Underwood 1997) and to incorporate inter-individual variability in the analyses. The three repeated experiments were analyzed separately. For each variable tested, assumptions of normality were tested using the Shapiro-Wilks procedure and the homogeneity of variance was analyzed by examination of residual vs. predicted values for each response variable. Data were log-transformed ($\log(x+1)$) if necessary. The Student-Newman-Keuls (SNK) or Least Significant Differences (LSD) tests were used to compare means when significant ($p < 0.05$) main effects were detected. All statistical

analyses were performed using Statgraphics version XV. Significant ANOVA results are reported and denoted within the text by their p-value and their F-ratio. Factor and error degrees of freedom are indicated with each F-ratio value.

Clam Experiments

Clam collection and maintenance.

Wedge clams (*Rangia cuneata*) were collected from the upper portion of the Caloosahatchee Estuary near Beautiful Island (see Figure 1). The salinity at the time of collection (February 2005) was 2 parts per thousand (ppt). After collection clams were placed in 10 gallon aquaria under aerated, static conditions. Prior to the start of the experiment clams were acclimated for 4 weeks at 3-4 ppt and fed 0.1g/algal paste/ individual/day.

Laboratory sediment exposures

Clam sediment exposures were carried out in a static renewal system for 30 days, with water changed every 3 days. Commercially available (Ward's) clay (< 4 μM) and silt (40-60 μM) sediments were used for sediment exposures. A total of two test doses (1 and 2 g sediment per individual clam) plus a control were used for two sediment sizes, silt and clay. Clams were sampled at 0, 10, 20 and 30 days. Initially, 20 clams / dose / replicate were placed in a 10-gallon aquarium with 4 replicate tanks / sediment (clay or silt) / dose (0, 1 or 2 g) / sampling day (0, 10, 20, 30 days – total of 24 tanks and 480 clams). Aquaria were dosed with 0 (control), 1, and 2 gm of clay- or silt-sized sediment particles/clam/day. On each sampling day, 5 clams were removed from each aquarium and dissected for shell height, shell weight, dry tissue weight, condition index, glycogen, and protein measurements. The total amount of sediment added to each aquarium was adjusted to the number of clams remaining in each tank after sampling to maintain a constant dose (1 or 2 g) per individual.

Wet and dry tissue weight measurements

Tissues were removed from shells and placed in pre-weighed vials. The vials were then weighed to obtain wet weights to the nearest 0.001 g. Tissues in vials were then freeze-dried for 48 hours and promptly reweighed to obtain dry weights to the nearest 0.001 g.

Shell height and shell weight measurements

Shells were dried for 24 hours at 60 °C and weighed to the nearest 0.1 g. Shell height was measured from the anterior (shell hinge) to posterior (edge at the highest point) to the nearest 0.1 mm using vernier calipers.

Clam Condition Index

Condition index was determined as the ratio of dry meat weight to dry shell weight (Lucas and Beninger 1985).

Glycogen Analysis

Glycogen content of homogenized freeze-dried clam tissue was estimated using the anthrone reagent method according to Van Handel (1965) and Baturo et al (1995) as detailed in the previous section (oyster experiments).

Protein Analysis

Total protein concentration was determined by a modified Lowry protocol (DC BioRad assay, Lowry 1951). An aliquot of the homogenate prepared for glycogen analysis was removed and diluted 1:3 in 1.0 M NaOH. Diluted samples were boiled in water for 5 min and assayed for protein content on 96 well microplates at 690 nm. Protein amounts were expressed in mg/g dry weight.

Statistical analyses:

Data were analyzed as a mixed model ANOVA using sediment type (clay vs. silt) and dose (0, 1 and 2 g) as fixed factors and sampling time (0, 10, 20 and 30 days) as a random factor. Data were first analyzed for normality (Shapiro-Wilks test) and homogeneity of variance. Homogeneity of variance was analyzed by examination of residual vs. predicted values for each response variable. Data were log-transformed ($\log_{10}(x+1)$) if necessary. The Tukey test was used to compare means when significant ($p < 0.05$) main effects were detected.

Results

In situ sediment loads

A comparison of sediment loads among the 5 collection sites showed some variation due to site when averaged across the three sampling periods (1. November 25, 2003 – December 23, 2003 2. December 23, 2003 – January 26, 2004 3. January 23, 2004 – February 23, 2004). Cattle Dock had the highest overall sediment loads at 85.09 ± 37.61 (standard error) g dry weight. The next highest sediment loads were at Iona Cove (64.42 ± 23.18 g dry weight), Tarpon Bay (43.54 ± 4.78 g dry weight), Bird Island (21.98 ± 3.74 g dry weight) and Kitchel Key (17.59 ± 4.78 g dry weight) (Figure 2). Examining each site over the successive sampling periods shows that higher and more variable sediment loads at Cattle Dock from January 23 to February 23, 2004 accounted for the overall higher sediment loads at that site (Figure 3). Similarly, from November 25 to December 23, 2003 sediment dry weights were highest at Iona Cove, which accounted for the overall sediment load at that site. Other than those two examples, no apparent variation in sediment loads was observed over the three sampling periods across all sites. It should be noted that sediment traps were lost over the 2nd and 3rd samplings at several sites, resulting in some missing data (Table I). At Kitchel Key all 3 replicate sediment traps were lost over the Jan. 23 – Feb. 23, 2004 sampling. Because of missing replicates, data were not analyzed statistically.

Oyster Experiments

Dry weight and condition index

Experiment I

After 5 weeks of exposure to silt and clay sediments dry tissue weights of oysters decreased significantly by dose ($p=0.0249$ $F_{df=2,168} = 7.28$; See Table 2 for ANOVA results). The Student-Newman-Keuls (SNK) test for multiple comparisons showed that dry weight significantly decreased between the 0 gram (g) and 3.0 gram (g) sediment doses ($p<0.05$, See Figure 4). Sediment type did not affect dry weight. Interactive effects between sediment type and dose also did not significantly affect dry weight.

ANOVA detected a significant effect on condition index in a similar manner to that of the dry weights. Condition index also decreased significantly with sediment dose ($p=0.0125$ $F_{df=2,152}=9.90$; Table 3). Condition index was significantly lower in 3.0 g doses compared to 0 and 1.5 g doses across both sediment types ($p<0.05$, SNK test; Figure 5). As with dry weight, effects of sediment type and interaction effects (sediment type x sediment dose) did not significantly affect condition index.

Experiment II

Dry weights were not significantly affected by the main effects of sediment type and dose nor their interactive effects (see Table 4). Condition index values were log transformed (\log_{10} Condition Index +1) to meet assumptions of normality for ANOVA. Condition index was also not significantly affected by sediment type or dose. Interactive effects did significantly affect condition index, however ($p<0.00001$, $F_{df=2,168}=15.89$; Table 5). Condition index decreased with increasing doses of silt, while condition index increased from 0 g to 1.5 g in oysters exposed to clay sediments, and then decreased at 3.0 g clay sediment (Figure 6).

Experiment III

In Experiment III dry weights were not significantly affected by main effects of sediment or dose. Interactive effects, however, were significant ($p=0.0252$ $F_{df=2,168}=3.76$; Table 6). The significant interaction effect was represented by a graded increase in dry weight from 0 to 3.0 g of clay sediment exposure, while dry weights were decreasing under silt exposure from 0 to 1.5 g. (Figure 7). Condition index was not significantly affected by either main or interactive effects (Table 7).

Glycogen content

Experiment I

Log transformed (\log_{10} glycogen +1) glycogen contents were significantly lower in oysters exposed to silt compared to clay-exposed oysters ($p=0.0005$, $F_{df=1,168}=12.76$; Table 8 and Fig. 8). Unlike dry weight and condition index, glycogen content was not significantly affected by sediment dose. Tank effects were significant, however ($p=0.0006$, $F_{df=6,168}=4.20$) and reflected an increase in glycogen contents from tank 1 to tank 3 (data not shown).

Experiment II

As with condition index, interactive effects significantly affected log transformed glycogen content in oysters ($p<0.00001$, $F_{df=2,168}=11.53$; Table 9). However, trends showed a contrary effect as glycogen contents were higher in silt-exposed vs. clay-exposed oysters at 0 and 1.5 g, but were lower in silt-exposed oysters at 3.0 g (Fig. 9). Main effects of sediment and dose were not significant, although there was a trend of increasing glycogen at 1.5 g sediment exposure ($p=0.062$).

Experiment III

In Experiment III, effects of sediment dose on log transformed glycogen were significant ($p=0.0426$, $F_{df=2,168}=5.59$; Table 10). A LSD test showed that sediment doses of 3.0 g resulted in decreased glycogen content compared to 0 and 1.5 g doses ($p<0.05$, Fig. 10). Other factors did not significantly affect glycogen content.

Clam Experiments

Shell height and shell weight

Shell height was not significantly affected by the main factors of sediment, dose or sampling day. There was an interactive effect between sediment and dose, however ($p=0.0414$, $F_{df=6,72} = 4.66$; Table 11). Shell weight did not change significantly due to main or interactive effects.

Dry weight and condition index

Condition index declined significantly with sampling date ($p=0.0084$, $F_{df=3,72} = 14.62$; Table 12). At 20 and 30 days the decrease in condition index began to decline significantly from the initial sampling (day 0) (SNK test $p<0.05$ for day 0 vs day 20 and day 0 vs day 30; see Fig. 11). The decrease in condition index was reflected by the decrease in dry weight which also significantly decreased with sampling day ($p=0.0177$, $F_{df=3,72} = 7.19$; Table 13). A significant interaction was also seen between sediment dose and sampling day ($p=0.0416$, $F_{df=6,72} = 4.66$). For the effect of sampling day alone, again significant differences were observed when comparing day 0 vs day 20 and day 0 vs day 30 (SNK: $p<0.05$; Fig. 12).

Glycogen and protein

Mean glycogen values were log-transformed to meet assumptions of normality. No significant changes in log-transformed glycogen were observed due to main effects of sediment type, dose or sampling day. There was a significant interactive effect between sediment and day ($p=0.0442$, $F_{df=6,72} = 5.05$; Table 14 Fig. 13). Protein also did not significantly change with main effects. There was, however, a significant interactive effect between sediment, dose and sampling day ($p=0.0064$, $F_{df=6,72} = 3.30$).

Discussion

Urbanization of the Caloosahatchee Estuary has significantly altered its salinity environment. Whether these anthropogenic alterations have been intentional, such as the diversion of freshwater from Lake Okeechobee to the Caloosahatchee, or the result of increasing residential and commercial use, increased freshwater input has adversely affected the biota in this system and has brought with those releases associated impacts. Among those associated impacts is the increase in sediment loads. Increased sediment loads may be from re-suspension after high flow events or due to commercial activities such as dredging. The purpose of this study was to examine the potential effects of high sediment loads on two key bivalve species in the Caloosahatchee. These two species (*Crassostrea virginica* and *Rangia cuneata*) may serve as important indicators of the effects of physical changes to this important estuarine system.

Oyster Sediment Experiments

Effects of different doses of clay and silt were variable on measures of oyster health (dry weight, condition index and glycogen content). Dry weight and condition index exhibited similar trends in Experiment I, in which dry weight and condition index decreased significantly at the highest sediment doses. However, in Experiment II and III no significant effects of either sediment type or dose on dry weight or condition index were detected. Significant interactive effects were detected in Experiments II (condition index) and III (dry weight), but results did not indicate a distinct pattern. Variable effects between experiments were also seen in glycogen contents. Silt exposed oysters had lower glycogen content than clay-exposed oysters in Experiment I, but in Experiment III

glycogen was more affected by dose with decreased levels of glycogen at 3.0 g exposure. In Experiment II only interactive effects were significant. However, the lowest glycogen amounts were in oysters exposed to 3.0 g silt, indicating that as in Experiment I silt induced a greater stressful effect than clay.

The amounts of sediments used in these experiments were nominal doses and actual exposure to each oyster may have been variable due to several potential factors. First, because these were nominal doses of sediment, actual amounts of suspended sediment may have varied due to differences in aeration between tanks. Filtration rates may have also varied between individual oysters resulting in different levels of exposure. This potential variation in the amount of sediment exposure may account for variation in the oyster performance measurements. Sediment concentrations in coastal waters typically range from a few mg L^{-1} to hundreds of mg L^{-1} (Oviatt and Nixon 1975; Biggs 1978; Peddicord 1977). Although the sediment concentrations used in these experiments were much higher ($\sim 0.4 \text{ g L}^{-1}$ (1.5 g/oyster) to $\sim 0.8 \text{ g L}^{-1}$ (3.0 g/oyster)), the actual exposure amounts may have been much lower. Increased sedimentation at moderate levels is not detrimental to some organisms. Sediment concentrations below 0.1 g L^{-1} actually enhanced larval growth rate in several bivalve species (Davis and Hidu 1969; Grant et al. 1990). This effect was speculated to be caused by increased levels of microflora made available by mixing events and would be relevant in nature, but not under the laboratory conditions described in this study. The sediments used in these experiments were likely devoid of any significant organic content as they were purchased from a commercial supplier.

It is also possible that a lower overall condition in oysters maintained in the laboratory may have made condition index a less sensitive measurement of oyster health. Condition index steadily declined in oysters from Experiment I to III. In Experiment I the average condition index (2.4) of oysters was similar to values typical of their site of collection (range of ~ 2.5 -2.8 at Bird Island). Average oyster condition index in Experiment II and III was 1.8 and 1.4 respectively. This decrease in condition index between experiments may have reflected declining nutritive conditions as oysters were held longer under laboratory conditions. In Experiment III the main factors of sediment and dose may have had little influence as condition was already low.

Glycogen is the primary carbohydrate store in mollusks and is important in providing energy during gametogenesis. Glycogen is also the first utilizable substrate during stress. The implications of sediment stress, or any external stress for that matter, are that increased stress will reduce the ability of an oyster to successfully reproduce as more glycogen is used to support metabolic functions necessary for survival under stress. The effects of sedimentation on glycogen in oysters were also variable. Increased doses of sediment lowered glycogen content in Experiment I but had no effect in subsequent experiments. As with condition index, glycogen contents were also significantly lower in Experiment III. However, glycogen served as a more sensitive indicator of sediment effects as silt exposure significantly lowered glycogen content compared to clay.

Clam Sediment Experiments

Condition index did not appear to be significantly affected by sediment type or level of exposure. As shell weight was not significantly different between treatments, the changes in condition index were related to primarily to changes in dry weight. However, changes in condition index and dry weight were not due to effects of sediment type and dose but decreased significantly over

successive sampling days. The sequential decrease in condition index may represent the effects of holding conditions despite consistent water changes and daily feedings. Food quality may have been an issue as clams were fed a commercial algal paste which may not adequately simulate the natural food environment of *Rangia*. Regardless, the sediments and the amounts administered did not significantly affect clam physiology. Sediment loads in the wild may be much higher and future experiments should incorporate a broader range of sediment loads. Previous studies in the field had found that substrate type was correlated with changes in condition index of *R. cuneata*. Mortality, condition index and growth were all adversely affected by clay-silt sediments compared to sand in clams from Pamlico Sound in North Carolina (Tenore et al. 1968). An inability to burrow in compact clay-silt substrate to avoid predation and high organic content causing low oxygen were cited as potential causes of these effects. Changes in factors associated with water overlying either sand or mud (~80% silt-clay) was implicated in affecting the condition index of *R. cuneata* in the James River, Virginia (Peddicord 1977). In this study field sites that consisted of mud had much higher levels of suspended sediments (range of 100-700 mg L⁻¹) than sandy sites, which likely contributed to the lower condition index of clams in mud (Peddicord 1977).

The use of condition index as a sensitive physiological indicator was promising as it detected very small differences in condition. Much of this had to do with the relative uniformity of size of the collected clams. The availability of a large number of uniformly sized individuals, leading to low inter-individual variability will make this species a useful subject for future physiological tolerance experiments. Glycogen values, however, displayed a high level of inter-individual variability, making differences between treatments difficult to discern. There did appear to be an overall decrease in glycogen from day 0 in clams treated with silt only and a decrease in clay-treated clams at day 30. Apparent differences were not significant, however.

Implications of Excessive Sedimentation in the Caloosahatchee River Estuary

Sediment loads were measured at 5 sites within the Caloosahatchee River that are currently monitored for effects of freshwater releases on oyster reefs. The resuspension of sediments due to increased freshwater flow may adversely impact oyster health and thus sediment loading should be considered as an important variable to measure along with other physical parameters such as temperature, salinity and dissolved oxygen. Site-specific differences in sediment loads may contribute significantly to differences in biological parameters between sites. Although the measurements in this study may be considered preliminary, there appears to be some apparent differences in sediment loads between sites. Cattle Dock had the highest sediment loads, although much of that was attributed to one month's sampling. The Cattle Dock site has been characterized as having relatively higher anthropogenic influence as it receives runoff from the city of Cape Coral and is located near several marinas, resulting in lower water quality and higher current flow (Volety et al. 2003). Performance measures of oyster health were also found to be lower compared to the more downstream sites of Bird Island, Kitchel Key and Tarpon Bay in previous studies. Condition index, growth of juvenile oysters and spat recruitment were all lower at Cattle Dock compared to the 3 aforementioned downstream sites (Iona Cove was not included in the previous study) (Volety et al. 2003). Although much of these differences are attributed to lowered salinity, the additive effects of increased sedimentation as a result of increased freshwater flow should not be discounted. Increased levels of suspended sediments could reduce pumping rate in oysters (Loosanoff 1948; Loosanoff and Tommers 1948; Loosanoff 1962). Along with reduced salinity, increased sedimentation may also reduce oyster recruitment as oyster settlement is higher on

shells with less siltation (Mackenzie 1983). High levels of suspended silt could also interfere with the feeding apparatus of swimming stages of oyster larvae (Carriker 1986).

The most recent study on sediments in the Caloosahatchee surveyed physical and chemical characteristics of bottom sediments in the Caloosahatchee river and estuary (Fernandez et al. 1999). Although the focus of the survey was on sediment-associated contaminants and nutrients studies such as this may be invaluable for future field studies as many of the survey sites in Fernandez et al. 1999 overlap with the field sites described in this report and in Volety et al. 2003. In nature contaminants and nutrients associated with excess sedimentation would place an even greater stress on the physiology of oysters and clams. The combination of sediment and polychlorinated biphenyl (PCB) compound exposure reduced glycogen content in the adductor muscle of *C. virginica* (Encomio and Chu 2000). Exposure to PAH-contaminated sediments increased levels of stress proteins in *C. virginica* (Cruz-Rodriguez and Chu 2002). Contaminated sediments can also depress immunological function in oysters and increase susceptibility to oyster parasites such as *Perkinsus marinus* (Chu 1999; Chu et al 2002).

Sediments may also harbor pathogens that can be released into the water column during re-suspension. The presence and growth of *Vibrio vulnificus* was higher in sediment samples compared to water samples collected from sites within the Charlotte Harbor estuary (Lipp et al. 2001). Pathogens may increase in prevalence and intensity due to decreased host condition, as a result of sedimentation-related stress. Oysters from the Cattle Dock site had the highest *Perkinsus marinus* intensities over a two year period from 2001 to 2002 (Volety et al. 2003). Decreased host condition, as a result of stress (sedimentation, low dissolved oxygen and poor food supply) was hypothesized to be the reason for increased *P. marinus* infections in oysters located at the bases of artificial reefs (Lenihan et al. 1999). Oysters located at higher tidal heights may experience less exposure to parasites and greater access to food resulting in better condition and lower *P. marinus* infections (Lenihan et al. 1999). Increased disease susceptibility to two protozoan parasites *Perkinsus marinus* and *Haplosporidium nelsoni*, was documented in oysters near the sediment (living at the base of the reef) compared to those at the top of reef. Increased sediment concentration and low dissolved oxygen were speculated to be the reasons of increased disease susceptibility (Volety et al. 2000). It is possible that inherent *P. marinus* infections may have affected the physiology of *C. virginica* and confounded effects of sediments alone in these lab experiments. However, although infection can affect oyster condition, the infection levels of oysters collected from Bird Island and other sites in the Caloosahatchee River are generally not high enough to detect deleterious sub-lethal effects. Energetic depletion and discernible effects on host physiology are estimated to occur only when infection intensities are heavy (Choi et al. 1989). The average infection intensities of oysters from Bird Island were considered to be very light, or low (Volety et al. 2003). It is possible that infections intensified in oysters during acclimation and the experiment. Infections by *P. marinus* were not determined however. Sediment effects on development of *P. marinus* infection within the oyster should be examined, particularly since additional stressors, such as contaminants can exacerbate *P. marinus* infections in oysters (Chu and Hale 1994).

Identifying regions within the Caloosahatchee of varying sedimentation has important implications on the restoration of oyster reef habitat in this estuary. Currently, artificial oyster reefs are deployed within the Caloosahatchee River in areas of favorable salinity and recruitment. Sedimentation rates may also affect the suitability of a particular site for restoration and may affect

the design of artificial reefs. Height of reefs, for example, may influence the nature of sedimentation upon restored reefs. Oysters suspended higher in the water column are less susceptible to the effects of sedimentation (McKinney and Case 1973). Increased sedimentation occurred on the bases of artificial oyster reefs and upon lower profile reefs resulting in decreased oyster survival, growth and condition (Lenihan 1999). Monitoring of sediment loads at proposed restoration sites before and after deployment of artificial reefs could potentially form part of the criteria to assess the success of oyster restoration within the Caloosahatchee or other areas in southwest Florida where these activities are being conducted.

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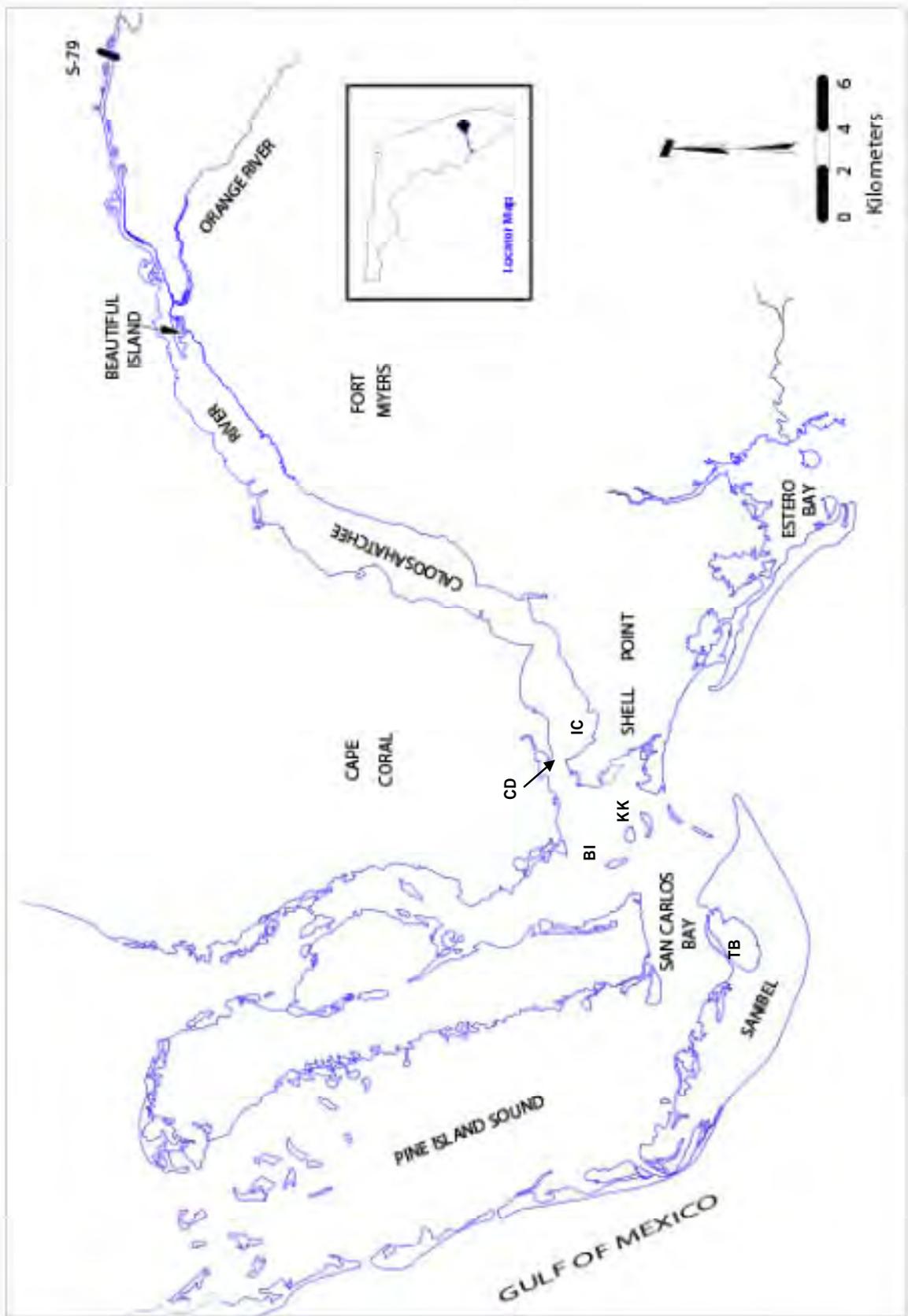
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Figure 1. Map of the Caloosahatchee River and estuary depicting Sampling sites for sediment traps and collection sites of oysters (Bird Island) and clams (Beautiful Island) for lab experiments. IC = Iona Cove, CD = Cattle Dock, BI = Bird Island, KK = Kitchel Key, and TB = Tarpon Bay. See Methods and Materials section for latitude and longitude locations. More Detailed site descriptions can be found in (Volety et al. 2003).



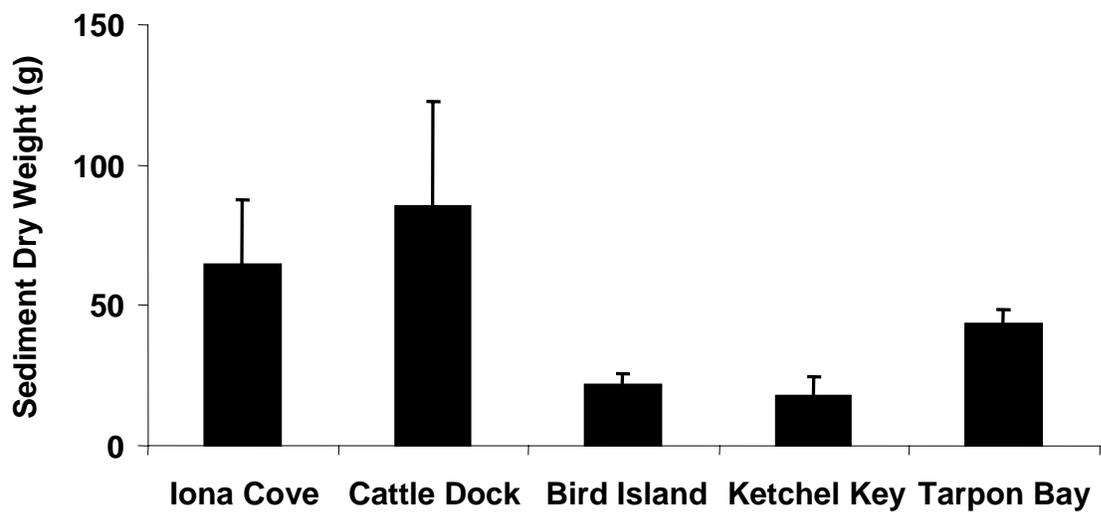


Figure 2. Sediment dry weights collected in sediment traps over a 3 month period at 5 sites within the Caloosahatchee River. Data are presented as mean sediment dry weights + standard error (SE). See Table I for n values.

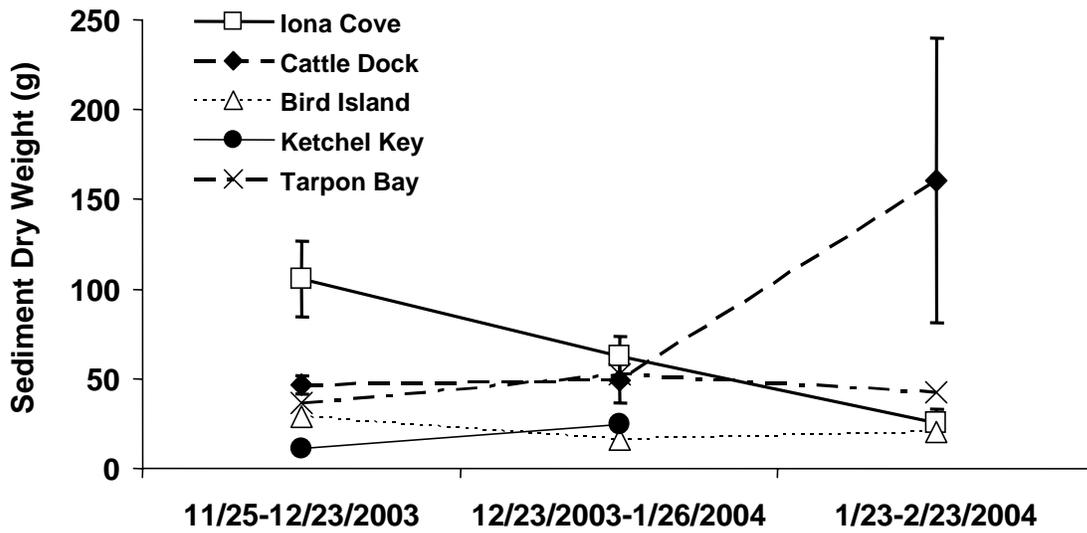


Figure 3. Dry weights of sediments collected at 5 Caloosahatchee sites as a function of sampling time. Data are mean sediment dry weights \pm SE.



Figure 4. Experiment I: Dry weights of oyster tissues exposed to various sediment amounts (0, 1.5 and 3.0 g / individual oyster). Data are mean dry weights + SE. Different letters indicate significantly different means (SNK test $p < 0.05$).

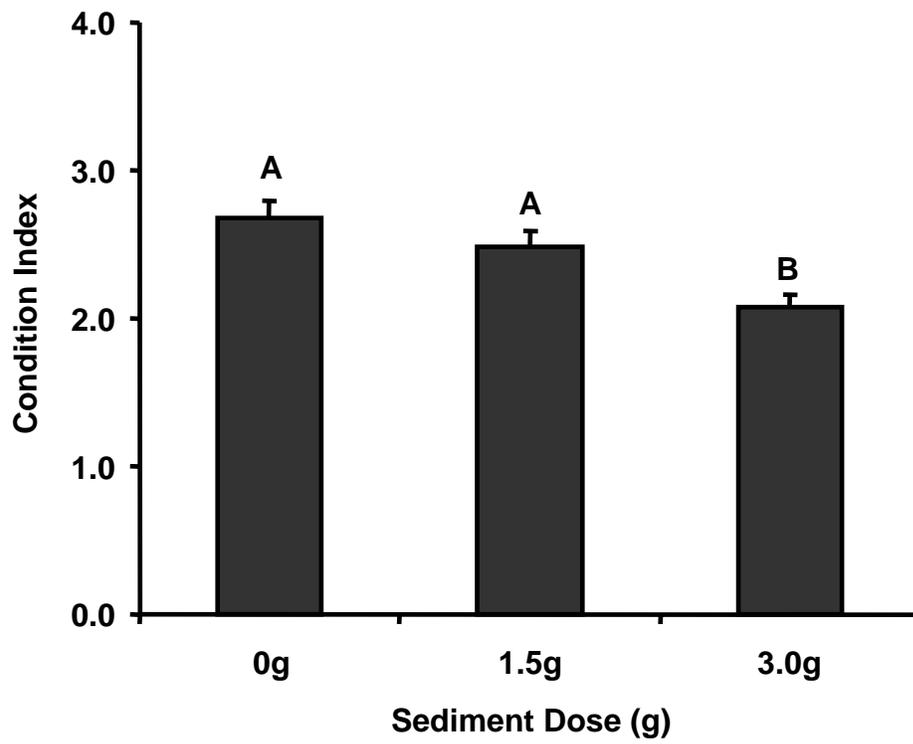


Figure 5. Experiment I: Condition index of oysters as a function of sediment dose. Data are mean dry weights + SE. Different letters indicate significantly different means (SNK test $p < 0.05$).

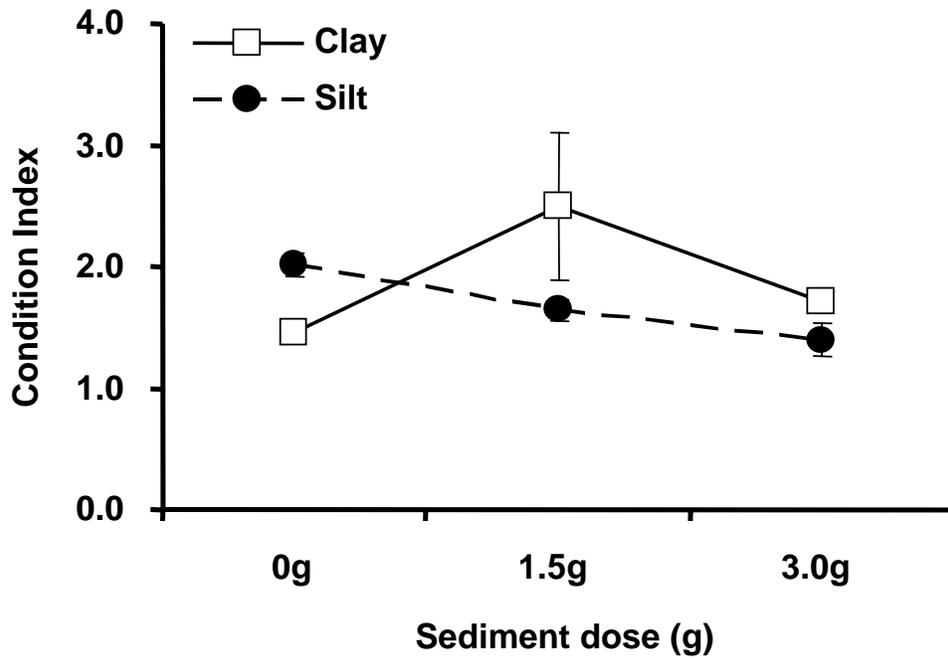


Figure 6. Experiment II: Condition index in oysters exposed to increasing doses of clay and silt. The graph depicts trends representing a significant interaction effect (sediment type x sediment dose) from ANOVA ($p < 0.00001$). Data presented are means \pm SE.

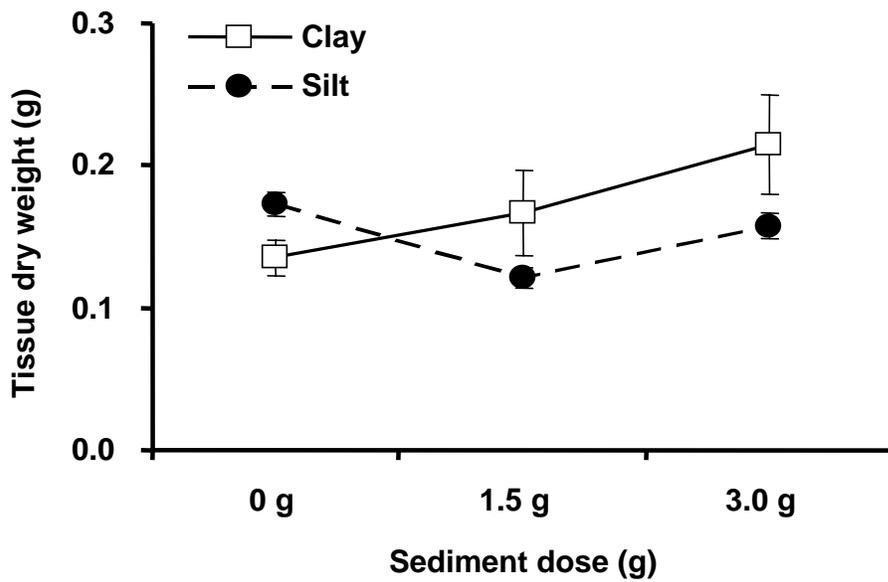


Figure 7. Experiment III: Oyster dry weights of oysters exposed to increasing doses of clay and silt. The graph depicts trends representing a significant interaction effect (sediment type x sediment dose) from ANOVA ($p=0.0252$). Data presented are means \pm SE.

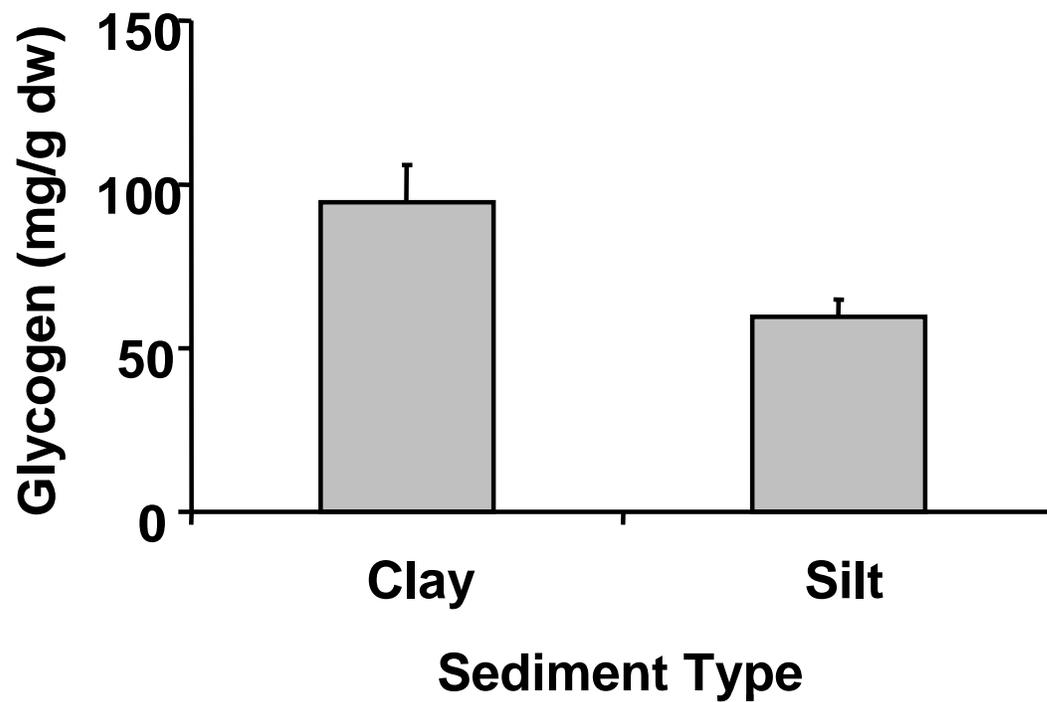


Figure 8. Experiment I: Glycogen contents in oysters exposed to clay and silt sediments. Glycogen was significantly lower in oysters that were exposed to silt compared to clay-exposed oysters (ANOVA: $p=0.0005$). Data are shown as mean glycogen (mg/ g dry weight (dw)) + SE.

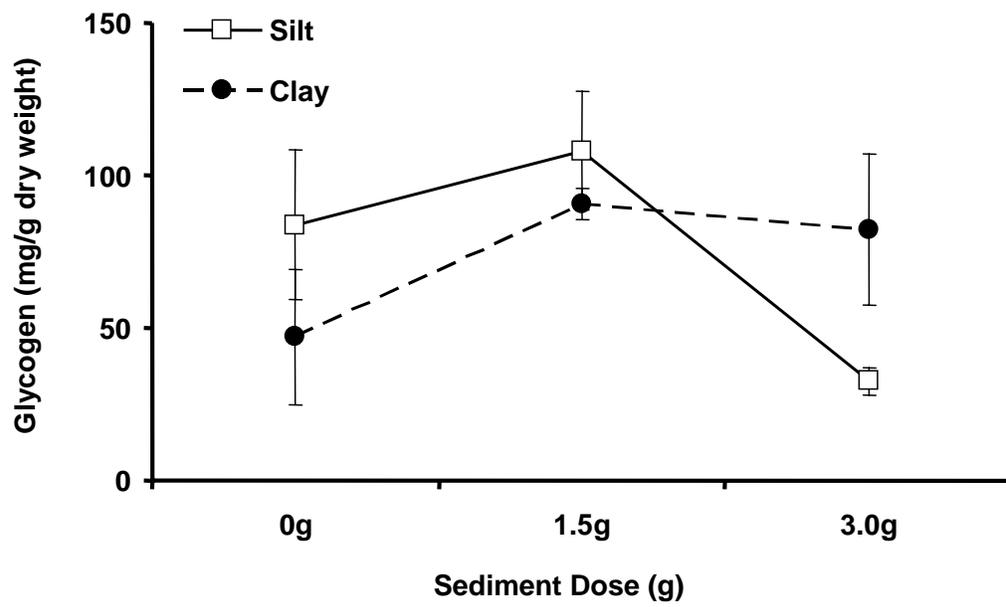


Figure 9. Experiment II: Interactive effects (sediment type x sediment dose) on glycogen contents in *C. virginica* (ANOVA: $p < 0.00001$). Data are mean glycogen contents \pm SE.

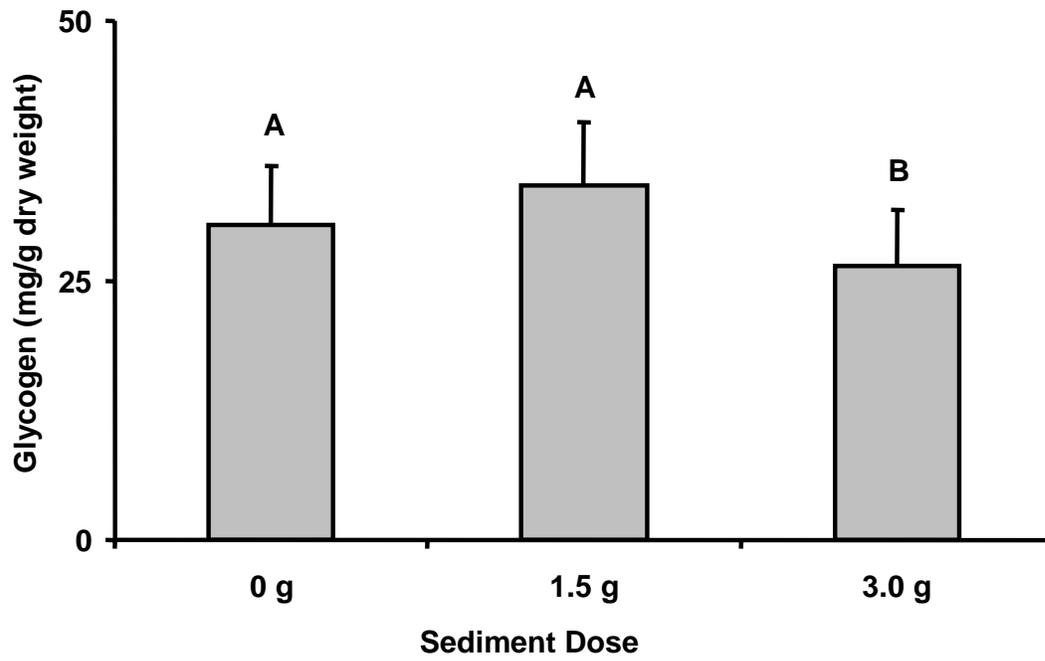


Figure 10. Experiment III: Effects of sediment dose on glycogen in oysters. Significantly different means (SNK test $p < 0.05$) are indicated by different letters. Data are means + SE.

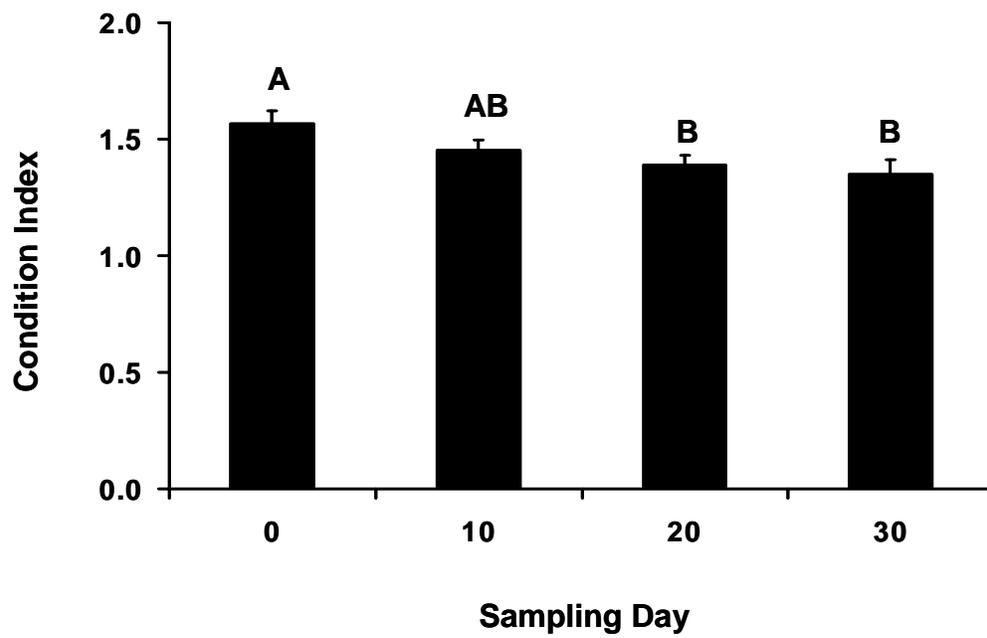


Figure 11. Condition index in *Rangia cuneata* as a function of sampling day. Different letters indicate means that are significantly different (SNK test, $p < 0.05$). Data are means + SE.

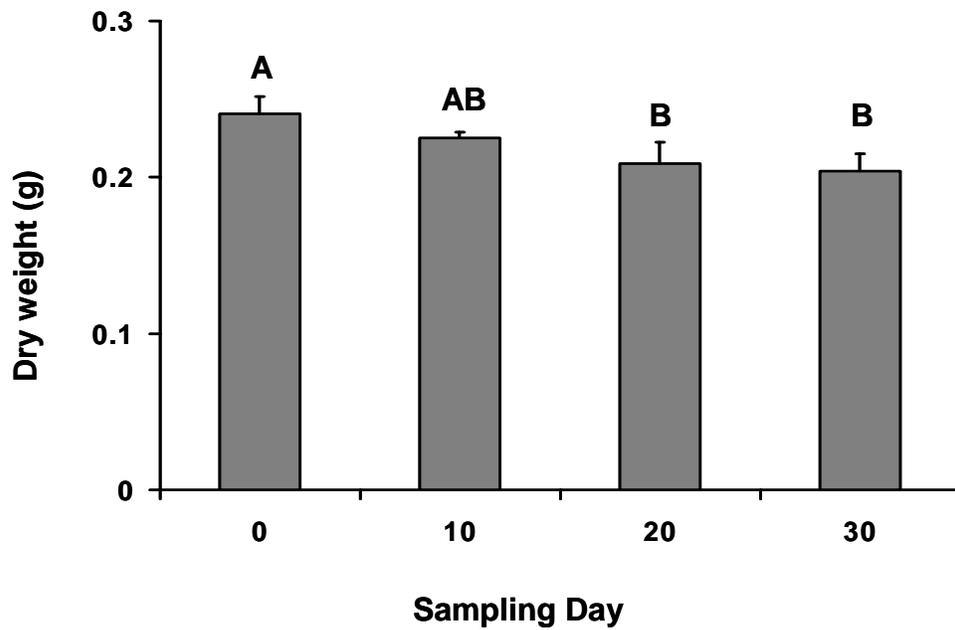


Figure 12. Total tissue dry weights in *Rangia cuneata* as a function of sampling day. Different letters indicate means that are significantly different from each other. Data are means + SE.

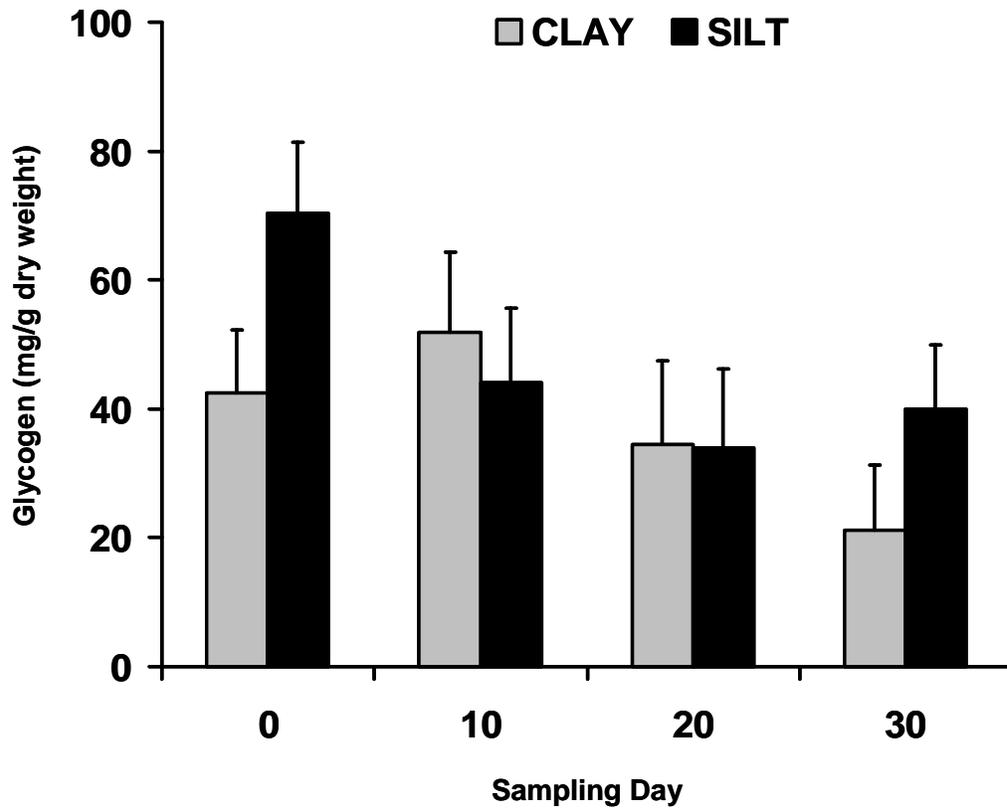


Figure 13. Glycogen contents in *Rangia cuneata* as a function of sampling day and sediment type. Interactive effects (sediment type x sampling day) were significant (ANOVA; $p=0.0442$). Data are means + SE.

Date	Replicate	Iona Cove	Cattle Dock	Bird Island	Kitchel Key	Tarpon Bay
11/25/2003- 12/23/2003	1	146.65 g	45.8 g	34.22 g	15.36 g	31.37 g
	2	93.71	38.22	30.19	12.30	44.42
	3	76.52	55	22.80	4.84	32.87
12/23/2003- 1/26/2004	1	67.49	60.98	19.62	40.71	71.45
	2	78.86	36.26	15.49	Trap lost	33.58
	3	40.33	Trap lost	13.99	19.60	Trap lost
1/26/2004- 2/23/2004	1	40.80	98.47	23.17	Trap lost	Trap lost
	2	14.86	64.17	Trap lost	Trap lost	35.37
	3	20.59	318.26	17.83	Trap lost	48.38

Table 1. Sediment trap data for 5 sites in the Caloosahatchee River. Sediment amounts for each replicate trap are reported as grams (g) dry weight. Replicate traps that were lost are indicated.

ANOVA Results - Oyster Sediment Experiment

Experiment I

Analysis of Variance for Dry Meat Weight

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	0.2578	11	0.02344	1.55	0.1176
Residual	2.5380	168	0.01511		
Total (Corr.)	2.7958	179			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.0004263	1	0.0004263	0.03	0.8668
Dose	0.1637	2	0.08187	7.28	0.0249*
Sediment*Dose	0.02618	2	0.01309	0.87	0.4224
Tank(Dose)	0.06750	6	0.01125	0.74	0.6145
Residual	2.5380	168	0.015107		
Total (corrected)	2.7958	179			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(5)+Q1
Dose	(5)+20.0(4)+Q2
Sediment*Dose	(5)+Q3
Tank(Dose)	(5)+20.0(4)
Residual	(5)

Table 2. Experiment I ANOVA results for effects of sediment type (clay or silt), sediment dose (0, 1.5 and 3.0 g/oyster/day) and interactive effects on dry tissue weight in the eastern oyster *C. virginica*. An asterisk (*) denotes a statistically significant result ($p < 0.05$).

Analysis of Variance for Condition Index

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	16.6143	11	1.5104	2.11	0.0225*
Residual	108.6770	152	0.7150		
Total (Corr.)	125.2910	163			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Dose	10.422	2	5.2110	9.90	0.0125*
Sediment	0.9007	1	0.9007	1.26	0.2635
Dose*Sediment	2.3611	2	1.1805	1.65	0.1953
Tank(Dose)	3.1564	6	0.5261	0.74	0.6215
Residual	108.677	152	0.7150		
Total (corrected)	125.291	163			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Dose	(5)+18.1394(4)+Q1
Sediment	(5)+Q2
Dose*Sediment	(5)+Q3
Tank(Dose)	(5)+18.1685(4)
Residual	(5)

Table 3. Experiment I ANOVA results for condition index. An asterisk (*) denotes a statistically significant result ($p < 0.05$).

Experiment II

Analysis of Variance for Dry Meat Weight

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	0.1328	11	0.01207	0.94	0.5006
Residual	2.1506	168	0.01280		
Total (Corr.)	2.2834	179			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.01188	1	0.01188	0.93	0.3369
Dose	0.03508	2	0.01754	2.05	0.2097
Sediment*Dose	0.03450	2	0.01725	1.35	0.2626
Tank(Dose)	0.05136	6	0.008559	0.67	0.6751
Residual	2.1506	168	0.01280		
Total (corrected)	2.2834	179			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(5)+Q1
Dose	(5)+20.0(4)+Q2
Sediment*Dose	(5)+Q3
Tank(Dose)	(5)+20.0(4)
Residual	(5)

Table 4. Experiment II ANOVA results for oyster dry weight.

Experiment II

Analysis of Variance for log (Condition Index +1)

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	0.5318	11	0.04834	5.38	<0.00001*
Residual	1.5093	168	0.008984		
Total (Corr.)	2.04103	179			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.01694	1	0.01694	1.89	0.1715
Dose	0.12050	2	0.06025	3.32	0.1069
Sediment*Dose	0.2855	2	0.1427	15.89	<0.00001*
Tank(Dose)	0.1089	6	0.01814	2.02	0.0657
Residual	1.5093	168	0.008984		
Total (corrected)	2.04103	179			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(5)+Q1
Dose	(5)+20.0(4)+Q2
Sediment*Dose	(5)+Q3
Tank(Dose)	(5)+20.0(4)

Table 5. Experiment II ANOVA results for condition index (log transformed). An asterisk (*) denotes a statistically significant result ($p < 0.05$).

Experiment III

Analysis of Variance for Dry Meat Weight

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	0.2294	11	0.020856	1.95	0.0361*
Residual	1.7944	168	0.01068		
Total (Corr.)	2.02379	179			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.02158	1	0.02158	2.02	0.1570
Dose	0.05744	2	0.02872	2.46	0.1657
Sediment*Dose	0.08040	2	0.04020	3.76	0.0252*
Tank(Dose)	0.06999	6	0.01167	1.09	0.3691
Residual	1.7944	168	0.01068		
Total (corrected)	2.02379	179			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(5)+Q1
Dose	(5)+20.0(4)+Q2
Sediment*Dose	(5)+Q3
Tank(Dose)	(5)+20.0(4)
Residual	(5)

Table 6. Experiment III ANOVA results for oyster dry weight. An asterisk (*) denotes a statistically significant result ($p < 0.05$).

Experiment III

Analysis of Variance for Condition Index

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	3.4539	11	0.3140	1.47	0.1469
Residual	35.8798	168	0.2136		
Total (Corr.)	39.3337	179			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.6817	1	0.6817	3.19	0.0758
Dose	0.7298	2	0.3649	1.79	0.2453
Sediment*Dose	0.8211	2	0.4106	1.92	0.1495
Tank(Dose)	1.2213	6	0.2036	0.95	0.4588
Residual	35.8798	168	0.2136		
Total (corrected)	39.3337	179			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(5)+Q1
Dose	(5)+20.0(4)+Q2
Sediment*Dose	(5)+Q3
Tank(Dose)	(5)+20.0(4)
Residual	(5)

Table 7. Experiment III ANOVA results for oyster condition index.

Glycogen

Experiment I

Analysis of Variance for log (Glycogen +1)

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	4.0212	11	0.3656	4.71	<0.00001*
Residual	13.0494	168	0.07768		
Total (Corr.)	17.0706	179			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.9910	1	0.9910	12.76	0.0005*
Dose	0.8045	2	0.4022	1.23	0.3560
Sediment*Dose	0.2682	2	0.1341	1.73	0.1810
Tank(Dose)	1.9575	6	0.3262	4.20	0.0006*
Residual	13.0494	168	0.07768		
Total (corrected)	17.0706	179			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(5)+Q1
Dose	(5)+20.0(4)+Q2
Sediment*Dose	(5)+Q3
Tank(Dose)	(5)+20.0(4)
Residual	(5)

Table 8. Experiment I ANOVA results for effects of sediment type and dose on glycogen content in oysters. Glycogen data were log-transformed to meet assumptions of normality and homogeneity of variance. An asterisk (*) denotes statistically significant results ($p < 0.05$).

Experiment II

Analysis of Variance for log (Glycogen +1)

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	5.1335	11	0.4667	3.61	0.0001*
Residual	21.7164	168	0.1293		
Total (Corr.)	26.8499	179			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.04188	1	0.04188	0.32	0.5700
Dose	1.2754	2	0.6377	4.58	0.0621
Sediment*Dose	2.98	2	1.49	11.53	<0.00001*
Tank(Dose)	0.8363	6	0.1394	1.08	0.3775
Residual	21.7164	168	0.1293		
Total (corrected)	26.8499	179			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(5)+Q1
Dose	(5)+20.0(4)+Q2
Sediment*Dose	(5)+Q3
Tank(Dose)	(5)+20.0(4)
Residual	(5)

Table 9. Experiment II ANOVA results for log-transformed oyster glycogen.

Experiment III

Analysis of Variance for log (Glycogen +1)

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	1.5423	11	0.1402	1.27	0.2440
Residual	18.5046	168	0.1102		
Total (Corr.)	20.0468	179			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.002188	1	0.002188	0.02	0.8881
Dose	0.7602	2	0.3801	5.59	0.0426*
Sediment*Dose	0.3717	2	0.1858	1.69	0.1882
tank(Dose)	0.4082	6	0.06803	0.62	0.7160
Residual	18.5046	168	0.1102		
Total (corrected)	20.0468	179			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(5)+Q1
Dose	(5)+20.0(4)+Q2
Sediment*Dose	(5)+Q3
tank(Dose)	(5)+20.0(4)
Residual	(5)

Table 10. Experiment III ANOVA results for log-transformed oyster glycogen.

ANOVA Results - Clam Sediment Experiment

Analysis of Variance for Shell Height

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	76.5499	23	3.3283	2.13	0.0081*
Residual	112.522	72	1.5628		
Total (Corr.)	189.072	95			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.02344	1	0.02344	0.04	0.8470
Dose	1.4233	2	0.7117	0.29	0.7553
Sampling Day	44.4303	3	14.8101	7.02	0.0503
Sediment*Dose	9.5275	2	4.7638	5.67	0.0414*
Sediment*Sampling Day	1.5912	3	0.5304	0.63	0.6211
Dose*Sampling Day	14.515	6	2.4192	2.88	0.1118
Sediment*Dose*Sampling Day	5.03917	6	0.8399	0.54	0.7781
Residual	112.522	72	1.5628		
Total (corrected)	189.072	95			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	$(8)+4.0(7)+12.0(5)+Q1$
Dose	$(8)+4.0(7)+8.0(6)+Q2$
Sampling Day	$(8)+4.0(7)+8.0(6)+12.0(5)+24.0(3)$
Sediment*Dose	$(8)+4.0(7)+Q3$
Sediment*Sampling Day	$(8)+4.0(7)+12.0(5)$
Dose*Sampling Day	$(8)+4.0(7)+8.0(6)$
Sediment*Dose*Sampling Day	$(8)+4.0(7)$
Residual	(8)

Table 12. ANOVA results for effects of sediment type (clay or silt), dose (0, 1 or 2 g/clam/day), length of exposure (sampling day – 0, 10, 20 or 30 days) and interactive effects on the shell height of the clam *Rangia cuneata*. An asterisk (*) denotes statistically significant results ($p < 0.05$).

Analysis of Variance for Condition Index

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	0.7876	23	0.03425	3.75	<0.00001
Residual	0.6579	72	0.009138		
Total (Corr.)	1.4455	95			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.01127	1	0.01127	1.06	0.3794
Dose	0.01865	2	0.009326	1.32	0.3357
Sampling Day	0.6502	3	0.2167	14.62	0.0084*
Sediment*Dose	0.01564	2	0.007820	2.69	0.1468
Sediment*Sampling Day	0.03196	3	0.01065	3.66	0.0826
Dose*Sampling Day	0.04250	6	0.007083	2.43	0.1516
Sediment*Dose*Sampling Day	0.01746	6	0.002910	0.32	0.9253
Residual	0.6579	72	0.009138		
Total (corrected)	1.4455	95			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(8)+4.0(7)+12.0(5)+Q1
Dose	(8)+4.0(7)+8.0(6)+Q2
Sampling Day	(8)+4.0(7)+8.0(6)+12.0(5)+24.0(3)
Sediment*Dose	(8)+4.0(7)+Q3
Sediment*Sampling Day	(8)+4.0(7)+12.0(5)
Dose*Sampling Day	(8)+4.0(7)+8.0(6)
Sediment*Dose*Sampling Day	(8)+4.0(7)
Residual	(8)

Table 13. ANOVA results for effects of sediment type, dose, length of exposure (sampling day) and interactive effects on the condition index of *R. cuneata*. An asterisk (*) denotes statistically significant results ($p < 0.05$).

Analysis of Variance for Dry Weight

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	0.02774	23	0.001206	1.72	0.0429
Residual	0.05051	72	0.0007015		
Total (Corr.)	0.07825	95			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.00009801	1	0.00009801	0.26	0.6482
Dose	0.0003702	2	0.0001851	0.27	0.7715
Sampling Day	0.01986	3	0.006620	7.19	0.0177*
Sediment*Dose	0.001283	2	0.0006416	4.38	0.0673
Sediment*Sampling Day	0.001152	3	0.0003839	2.62	0.1457
Dose*Sampling Day	0.004098	6	0.0006830	4.66	0.0416*
Sediment*Dose*Sampling Day	0.0008796	6	0.0001466	0.21	0.9729
Residual	0.05051	72	0.0007015		
Total (corrected)	0.07825	95			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(8)+4.0(7)+12.0(5)+Q1
Dose	(8)+4.0(7)+8.0(6)+Q2
Sampling Day	(8)+4.0(7)+8.0(6)+12.0(5)+24.0(3)
Sediment*Dose	(8)+4.0(7)+Q3
Sediment*Sampling Day	(8)+4.0(7)+12.0(5)
Dose*Sampling Day	(8)+4.0(7)+8.0(6)
Sediment*Dose*Sampling Day	(8)+4.0(7)
Residual	(8)

Table 13. ANOVA results for effects of sediment type, dose, length of exposure (sampling day) and interactive effects on the tissue dry weight of *R. cuneata*. An asterisk (*) denotes statistically significant results ($p < 0.05$).

Analysis of Variance for log (Glycogen +1)

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	2.6385	23	0.1147	5.18	<0.00001
Residual	1.5934	72	0.02213		
Total (Corr.)	4.2319	95			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	0.1966	1	0.1966	1.33	0.3328
Dose	0.1104	2	0.05522	0.79	0.4965
Sampling Day	1.1927	3	0.3976	2.11	0.2339
Sediment*Dose	0.09821	2	0.04911	1.67	0.2643
Sediment*Sampling Day	0.4445	3	0.1482	5.05	0.0442*
Dose*Sampling Day	0.4201	6	0.07001	2.39	0.1568
Sediment*Dose*Sampling Day	0.1760	6	0.02933	1.33	0.2572
Residual	1.5934	72	0.02213		
Total (corrected)	4.2319	95			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(8)+4.0(7)+12.0(5)+Q1
Dose	(8)+4.0(7)+8.0(6)+Q2
Sampling Day	(8)+4.0(7)+8.0(6)+12.0(5)+24.0(3)
Sediment*Dose	(8)+4.0(7)+Q3
Sediment*Sampling Day	(8)+4.0(7)+12.0(5)
Dose*Sampling Day	(8)+4.0(7)+8.0(6)
Sediment*Dose*Sampling Day	(8)+4.0(7)
Residual	(8)

Table 14. ANOVA results on the effects of sediment type, dose, length of exposure and interactive effects on glycogen content in *R. cuneata*.

Analysis of Variance for Protein

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Model	303551.0	23	13197.9	7.71	<0.00001
Residual	123328.0	72	1712.89		
Total (Corr.)	426879.0	95			

Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Sediment	985.031	1	985.031	0.11	0.7574
Dose	26250.9	2	13125.4	0.78	0.4994
Sampling Day	112292.0	3	37430.5	1.89	0.2471
Sediment*Dose	3522.32	2	1761.16	0.31	0.7432
Sediment*Sampling Day	25818.3	3	8606.11	1.52	0.3016
Dose*Sampling Day	100812.0	6	16802.0	2.98	0.1051
Sediment*Dose*Sampling Day	33870.3	6	5645.05	3.30	0.0064*
Residual	123328.0	72	1712.89		
Total (corrected)	426879.0	95			

Expected Mean Squares

<i>Source</i>	<i>EMS</i>
Sediment	(8)+4.0(7)+12.0(5)+Q1
Dose	(8)+4.0(7)+8.0(6)+Q2
Sampling Day	(8)+4.0(7)+8.0(6)+12.0(5)+24.0(3)
Sediment*Dose	(8)+4.0(7)+Q3
Sediment*Sampling Day	(8)+4.0(7)+12.0(5)
Dose*Sampling Day	(8)+4.0(7)+8.0(6)
Sediment*Dose*Sampling Day	(8)+4.0(7)
Residual	(8)

Table 15. ANOVA results on the effects of sediment type, dose, length of exposure and interactive effects on protein content in *R. cuneata*.

