

**Salinity and Temperature Tolerance Experiments on Selected Florida Bay Mollusks**

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## **Salinity and Temperature Tolerance Experiments On Selected Florida Bay Mollusks**

The ultimate goal of the Comprehensive Everglades Restoration Plan (CERP) is to restore and preserve the unique ecosystems of South Florida, including the estuaries. Understanding the effect of salinity and temperature changes, beyond typical oscillations, on the biota of South Florida's estuaries is a necessary component of achieving the goal of restoring the estuaries. The U.S. Geological Survey has been actively involved in researching the history of the South Florida Ecosystem, to provide targets, performance measures, and baseline data for restoration managers. These experiments addressed two aspects of ecosystem history research: 1) determining the utility of using molluscan shells as recorders of change in water chemistry parameters, primarily salinity, and 2) enhancing our *in situ* observations on modern assemblages by exceeding typically observed aquatic conditions. This set of experiments expanded our understanding of the effects of salinity, temperature and other water chemistry parameters on the reproduction, growth and overall survivability of key species of mollusks used in interpreting sediment core data. Observations on mollusks, plants and microbes made as part of these experiments have further refined our knowledge and understanding of the effects of ecosystem feedback and the role salinity and temperature play in ecosystem stability. The results have demonstrated the viability of several molluscan species as indicators of atypical salinity, and possibly temperature, modulations. For example *Cerithium muscarum* and *Bulla striata* demonstrated an ability to withstand a broad salinity and temperature range, with reproduction occurring in atypically high salinities and temperatures. These experiments also provided calibration data for the shell biogeochemistry of *Chione cancellata* and the possible use of this species as a water chemistry recorder. Observations made in the mesocosms, on a scale not normally observable in the field, have led to new questions about the influence of salinity on the localized ecosystem. The next phase of these experiments; to calibrate growth rate and reproductive viability in atypical salinities is currently underway.

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#### **Acknowledgments**

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#### **INTRODUCTION**

The South Florida ecosystem is currently undergoing a massive ecosystem restoration effort, utilizing private institutions and local, state, and federal government agencies. The restoration efforts are being coordinated by the Comprehensive Everglades Restoration Plan (CERP). The primary goal of the CERP is to restore more natural freshwater flow through the south Florida ecosystem including quantity and timing of freshwater deliveries into the system, and maintaining water quality within the overall system. Over the past century, changes have occurred in freshwater delivery from the terrestrial Everglades to the estuaries of South Florida due to anthropogenic activities. The construction of dams, canals, and other water control structures, and the increases in population with the resulting land-use change and road construction have restricted or diverted the natural flow of water through the Everglades ecosystem (Light and Dineen, 1994). These changes to the terrestrial ecosystem have altered the natural variability of the seasonal salinity cycle of the estuaries, including Florida Bay, Biscayne Bay and the southwest coastal areas (Figure 1).

Since 1995, several USGS projects have focused on the ecosystem history of South Florida. The role of ecosystem history research is to determine natural cycles of change prior to human alteration of the environment, to identify the range of natural variation, and to attempt to separate natural change from anthropogenic change. Ecosystem history research functions on basic paleoecological principals, including the principal of uniformitarianism – the present is the key to the past. Therefore, a significant component of the research has involved studying the modern environment and the ecology of the living biota of South Florida. Information on the modern environment



Figure 1. Site map showing South Florida Region

is then used to interpret biotic assemblages found in sediment cores collected from around the region.

Understanding the history of an ecosystem over biologically significant periods of time is an essential component of restoration because, in order to establish realistic and obtainable performance measures and targets for restoration, it is critical to first determine how the system functioned historically. A better understanding of how human activities and natural cycles affect ecosystems can be obtained by observing potential cause and effect relationships *in situ*, forming hypotheses, and then testing these hypotheses in a laboratory setting, under closely controlled and monitored conditions. As long as laboratory conditions faithfully replicate natural *in situ* conditions, then the inverse becomes a valid approach; laboratory observations and experiments can generate new hypotheses that can be tested by field observations (Murray, et.al. 2004).

In 2002, a system of large aquaria (mesocosms) were constructed at the U.S. Geological Survey, Leetown Science Center (LSC) in Leetown, West Virginia, in order to conduct experiments that test our field hypotheses and reproduce scenarios commonly found in the estuaries and marine ecosystems of South Florida. These aquaria allow us to test the effects of natural stressors, for example, salinity and temperature extremes, on flora and fauna typical of South Florida's estuaries, and shallow marine basins. The results of salinity and temperature experiments on selected mollusks are the subject of this report. These experimental data expand our understanding of the living biota, and therefore, our ability to interpret the historical data from cores.

#### **The Natural Setting**

Florida Bay is a reticulate system of mud banks and small keys or islands predominantly covered by red mangrove (*Rhizophora mangle*) that enclose over 40 shallow basins or depressions (Figure 1). The water depth in these basins ranges between two and six feet, and the mudbanks are often emergent during low tides (McPherson and Halley, 1996). This network of banks and islands limits the tidal variation and exchange of water from basin to basin. Restricted exchange of water coupled with the limited influx of freshwater can cause areas within Florida Bay to have large fluctuations in salinity, temperature, and nutrient content. In addition, one basin can be very different in terms of salinity and temperature from adjacent basins. Regions within a basin also can experience rapid temperature changes, due to the summer heat and evaporation. As the evaporation increases, the salinity can increase significantly, especially in shallow, localized areas. Conversely, summer thunderstorms can drop temperatures and salinity in a very short period of time in localized areas.

The variability of salinity and temperature within the estuaries of South Florida is largely controlled by the seasonal patterns of a sub-tropical climate and by specific storm events. Throughout the South Florida region, the typical annual climate cycle consists of a cool, dry season and a hot, wet season. The winter months (December to February) constitute the cool, dry season, which has an average seasonal temperature of 70.33°F and an average rainfall of 5.32 inches (Figure 2A-B) in the Key West, FL area. During the hot, wet summer months (June through August) the average seasonal temperature is



Winter (Dec-Feb) 1896 - 2005 Average = 70.33 degF Winter (Dec-Feb) 1896 - 2005 Trend = 0.09 degF / Decade



Figure 2A. Winter temperature average for the Key West area of South Florida Graph from the NOAA website, http://www.ncdc.noaa.gov/oa/climate/research/cag3/cag3.html Last visited on 11/29/05



Figure 2B. Winter precipitation average for the Key West area of South Florida. Graph from the NOAA website, http://www.ncdc.noaa.gov/oa/climate/research/cag3/cag3.html Last visited on 11/29/05

83.26°F and the average rainfall is 12.79 inches (Figure 3A-B). Typically, winter months are characterized by reduced freshwater runoff in the near shore environments and drier conditions in the Everglades. This reduction in runoff and direct rainfall on the surface of the basin causes salinity to go up while temperature goes down. The warmer summer months give rise to common daily rain squalls and stronger events such as hurricanes can affect the entire region. These weather events can dump large amounts of freshwater both into the terrestrial Everglades and the estuaries, and this influx of rain water can reduce the salinity and temperature, in some cases dramatically.

Along the terrestrial margins of the estuaries, this seasonal variability is the primary driver of salinity and temperature fluctuations. Salinities along the terrestrial margins (Figure 4) vary from nearly fresh (4.8 ppt) from June through November, slowly increasing to nearly marine salinities (30.65 ppt) from December through May. The reticulate system of banks within Florida Bay can cause the salinity in restricted basins to exceed 40 ppt and temperatures to exceed 30° C. Figure 5 illustrates the variation of salinities in different basins. These cycles of salinity and temperature change create a rigorous environment for the organisms living there.

#### **Experimental Design and Purpose**

Experiments on live organisms began in 2002 to fulfill several purposes for ecosystem history research. First, a project was started in 2001 to examine the utility of using molluscan shells as recorders of change in water chemistry parameters, primarily salinity. This work is briefly summarized in Stamm and Wingard (2004) and will be the subject of future reports. In order to use shells as recorders of change, however, we needed to understand their life histories and their ecology. When did the shells grow – year round or seasonally? Did they grow continuously throughout their life span or did growth rates slow? Did growth stop during certain environmental events, such as high salinity or temperatures? Second, observations on living mollusks in the field were only obtained periodically. (For Florida Bay, generally two times per year, once in the wet season and once in the dry season.) Consequently, we frequently missed obtaining critical biotic data associated with salinity or temperature extremes tolerated by living mollusks. These data are necessary to better understand the effects of salinity, temperature and other water chemistry parameters on the reproduction, growth and overall survivability of key species of mollusks. Field data alone did not provide us with a complete proxy data set for interpreting the downcore assemblages we are analyzing as part of the Ecosystem History projects or for addressing questions associated with the shell chemistry study. .

In order to address the needs of ecosystem history research, several experiments were designed. The experimental systems were utilized in this study to quantify the mortality of selected mollusks due to extremes in salinity and temperature that have been recorded in Florida Bay (Figure 6). The recorded high and low values of salinity and temperature were used to set the limits for the experimental values. Early tank



Figure 3A. Summer temperatures for the Key West area of South Florida. Graph from the NOAA website, http://www.ncdc.noaa.gov/oa/climate/research/cag3/cag3.html Last visited on 11/29/05



Figure 3B. Summer precipitation for the Key West area of South Florida Graph from the NOAA website, http://www.ncdc.noaa.gov/oa/climate/research/cag3/cag3.html Last visited on 11/29/05



Figure 4. Salinity at the Little Madeira water monitoring site, Florida Bay,. Data compiled and graphed by Robert Stamm, USGS



Florida Bay Salinity



Figure 5. Salinity oscillations for the years 1989 to 2001. The blue band indicates typical marine salinities for the South Florida region. All locations are shown on the satellite map.



Years 1955-1995

Figure 6. Historical salinity data for Florida Bay. (Unpublished data set by M. Robblee, USGS. Graphed by Joel Hudley, USGS

experiments conducted in Reston, Virginia from August 2000 until January 2003 (three experimental attempts), were all determined to be unsuccessful due to power failures that caused the water chemistry collapse in the experimental systems.

This report describes the results of the experiments conducted in order to determine the tolerance of nine key molluscan species to a broad range of salinity and temperature. The results supplement our field data by allowing us to extend the minimum and maximum values observed in the field, and to determine mortality and reproductive viability under atypical conditions. These data provide improved proxy data for interpreting historical salinity patterns preserved in the faunal assemblages in the sediment cores examined for the ecosystem history research projects.

Additional experiments and observations are ongoing in habitats established in Florida Bay in the winter of 2001 (under Everglades National Park Study #EVER-00141; and Permits 2000-004; EVER-2001-SCI-0048; EVER-2002-SCI-0038; EVER-2003-SCI-0053; EVER-2004-SCI-0099). The habitats were specifically designed to test growth and survivability *in situ* over an extended period of time, under varying environmental conditions of the pelecypod, *Chione cancellata;* this species was selected as the test organism for shell chemistry studies. These habitats also provide a control for the overall health and success of the mesocosms at LSC, i.e. if the *C. cancellata* in both locations are equally healthy, then the mesocosms are an accurate replicate of Whipray Basin, Florida. Details of the habitat work will be published in a separate report, but relevant to the mesocosm experiments was the environmental data from the two habitats in Whipray Basin (Figure 7), near water monitoring station FB21. The water monitoring stations were established by Everglades National Park and South Florida Water Management District. The data from the water monitoring station provided a template for reproducing the natural water chemistry conditions in the mesocosms at Leetown Science Center.

Nine molluscan species, representing eight genera, were selected for the experimental trials. These species are commonly found in our modern site surveys and in the cores examined for the ecosystem history studies. In addition, these were species that were relatively easy to find and collect in the field, survive transport back to Leetown, and were not known to be particularly selective about ecological requirements such as food source, small fluctuations in water chemistry, or substrate.

#### **MESOCOSMS**

The experimental aquatic culture systems (mesocosms) at Leetown Science Center were designed to replicate mesohaline to hypersaline ecosystems typical of South Florida estuaries. Two large (System A 2750 gallons and System B 2000 gallons) mesocosms were designed to replicate communities of seagrass beds and more open bottom environments; these were used for observation, culture and an experimental control system (Figure 8). Experimental systems were allowed to equilibrate for six months before the actual experimental cycles began. This process allowed the microbial, algal, animal, and plant communities to become established and stabilized before experiments began. Eight separate systems (220 gallons each) were constructed for the experiments. Both the experimental and the control systems were designed so that multiples of any experiment could be conducted simultaneously within the same system.









Figure 7. Top, map showing location of habitats in Whipray Basin. Middle, Images of habitats, taken August 2004. Bottom, Image of habitat as originally installed



Figure 8. Schematic of the Leetown Science Center Mesocosms, showing main systems and experimental systems.

The LSC mesocosms are composed of eleven separate flow-through circulating systems totaling more than eleven thousand gallons (Figure 9A-B). The mesocosms are situated within a 20,000 square foot greenhouse, which provides natural sunlight and a solar and lunar cycle similar to that found in Florida Bay. The systems at the LSC are monitored for water chemistry parameters, temperature, salinity, and light intensity (Figure 10) and these data are compared with data from water monitoring stations and other modern monitoring sites in Florida Bay. The combination of modern sampling site data and high frequency observations made at the Whipray habitats provide a scale by which the overall health of the LSC mesocosms can be measured and monitored.

Each system has a large sump tank, which is utilized as a refugium. A refugium tank is commonly used in the aquaculture industry for several reasons; protection from predation, increase the overall water volume, food source cultivation, plant growth for natural nitrate reduction, etc. The refugia tanks in the LSC system serve multiple functions. 1) These tanks provide a buffer for the system by increasing the overall volume of water so that any changes in water chemistry, temperature, etc. would be gradual and not create unwanted stress on the organisms. 2) When adding water to compensate for evaporation, and/or adding chemical components that are taken up by the marine organisms, the sump tanks provide a mixing and equilibration area so that dramatic changes to the system are reduced. 3) They provide an integrated part of the system where any monitors, control instruments, bio-filters etc. can be placed and not have a direct effect on organisms in the habitat part of the systems.  $\frac{1}{1}$  4) The refugia add stability because water parameter changes are more subtle as the overall mesocosm volume increases. The effect of unexpected problems such as pump and temperature control system shut-downs due to power outages is reduced with a large refugium. The only mechanical filters on the mesocosms are small protein skimmers that are used to remove heavy proteins and provide better gas exchange. The system is primarily maintained through natural bio-filtration carried out by the plant and animal assemblages in each system. The systems were designed in a gravity flow layout so that any power failure effects were minimized and no water loss would occur. This layout also allows the system to self start when power is restored.

The substrate in all systems is naturally occurring *Halimeda* hash (Figure 11). This medium provides a large surface area for microbial communities, which are necessary for the nitrogen-reduction cycles. *Halimeda* hash is biogenically created so as it dissolves it provides the trace-elements and metals (calcium, magnesium, strontium, etc.) typically present in seawater. These components are necessary for calcium carbonate production during growth of the organisms. In addition, the calcium carbonate composition of the *Halimeda* hash provides a pH buffer for the systems.

In order to provide adequate food, commercially available phyto-plankton and zooplankton mixtures are added to the tanks as food sources for the suspension feeding

organisms, but they have inadvertently become part of the biological system as plants or

animals in the main tanks reproduced and the offspring spread into the sump tanks.

<sup>&</sup>lt;u>1</u>  $1$  Originally, the sump tanks were not intended to contain any of the study



Figure 9. View showing tanks in the greenhouse at the LSC



Figure 10. Comparison of Leetown Science Center (LSC) mesocosms with Whipray Basin (WB) Salinities and temperatures. Whipray Basin data is combination of USGS and SFWMD monitoring data.



Figure 11. Picture of LSC tank floor showing substrate and various organisms. Photo by JBM

organisms in the system. Establishing epiphytic growth on the tank walls and plant assemblages was essential prior to starting any experimental studies because this provides the primary food source for grazing animals.

The mesocosms were monitored for water chemistry parameters using both wet chemistry methods and electronic instruments. Wet chemistry monitoring used commercially available titration kits to quantify strontium, calcium, iodine, alkalinity, and magnesium. The accuracy range was within 5-15 ppt for any metal measured depending on the titration method. Electronic probes were used to monitor salinity, temperature, pH, and dissolved oxygen; these were the same instruments used in the field to monitor *in situ* conditions. Temperature and light were monitored using separate data loggers that gather data in thirty minute intervals; these data loggers also were placed on the habitats in Whipray Basin (set at 180 minute intervals). These data gave a high resolution comparison of temperature and light between the laboratory and the *in situ* ecosystems (Figure 12).

The synthetic sea water used in the LSC mesocosms was made using multiple brands of common commercial aquaculture sea salt mixtures. Freshwater added to the commercial mixtures was a combination of deionized water and LSC well water. The well water has high calcium content and low nitrate levels so it was ideal for making synthetic sea water. Before adding newly mixed sea water to the systems, each batch was allowed to equilibrate so that pH changes would be subtle. New sea water was added to the refugia tanks so that the initial mixing was outside the habitat tanks. The fresh water used in the new sea water was a combination of deionized water and LSC well water.

Organisms were initially introduced into systems A and B at 35 ppt and 25ºC and were allowed to equilibrate for six months before experiments began. Four experiments were conducted simultaneously in four separate 220 gallon experimental systems:

- 1. System 1 experiments: Increasing salinity with relatively stable high temperature (at or above  $32^{\circ}$ C) -- analogous to summer conditions when evaporation exceeds rainfall.
- 2. System 2 experiments: Increasing salinity with relatively stable low temperatures (at or below  $17^{\circ}$ C) -- analogous to winter dry conditions when the humidity is low and winds cause accelerated evaporation. This is seen in historical NOAA (National Data Buoy System http://www.ndbc.noaa.gov/station\_page.php?station=LONF1 -) data for rainfall, wind direction and speed, and salinity.
- 3. System 3 experiment: Decreasing salinity with relatively stable high temperature (at or above  $32^{\circ}$ C) – analogous to summer conditions during a wet year when rainfall exceeds evaporation or when freshwater input is driven by rainfall in adjacent regions. This added freshwater provides a point source (canal mouth) stress to the organisms in the region.
- 4. System 4 experiment: Decreasing salinity with relatively stable low  $t$ emperatures (at or below 17 $^{\circ}$ C) -- analogous to winter season when rainfall equals or exceeds evaporation, and point source influence during the cooler winter months.

Salinity and temperature was altered gradually, in each experimental system from the starting point of 35 ppt and 24 °C over a period of 90 days and maintained at that level until none of the original test mollusks were remaining alive (Table 1). A control tank of



Figure 12. Comparison of Leetown mesocosm temperature to Whipray Basin water temperatures.

the same design and size as the experimental tanks was housed within system A (Figure 8) and was maintained at average marine salinity (32-35 ppt) and temperature (24-26 $^{\circ}$ C).

#### **RESULTS**

The experiments began on September 23, 2003 and continued for 210 days ending on April 19, 2004. At that time none of the original individuals from nine species, (eight genera), remained alive in the experimental systems, however there were individuals alive in the control system at this time. The systems were then maintained at the final salinity and temperature so that follow-up observations could be made on any offspring that were noted. Table 1 shows the duration of the experiments, and the final salinities obtained when all starting individuals were dead. Table 2 shows the duration of groups based on hyper/hypo saline systems. The following results are organized alphabetically by species and explain the response of each species to the four experiments. The salinity levels for each experiment are in parts per thousand (ppt) and all temperatures are in degrees Centigrade. The levels indicated are the final averages (FA), i.e. the, last ten days' average of the measurements, prior to the death of the last individuals (of a given species) in each experimental system. The high/low salinity systems are referred to as the hypersaline/hyposaline systems respectively.

*Brachidontes exustus*: *B. exustus* is commonly found throughout Florida Bay, in a wide range of salinities and temperatures, and is often seen in dense groupings. The individuals in the hypersaline, high temperature System 1 (FA 59 ppt and 31°C) survived for 181 days, with no juveniles observed. Individuals in the high saline low temperature System 2 (FA 57 ppt and 15°C) survived for 159 days and no juveniles were seen (Figure 13A). Individuals placed in the hyposaline, high temperature System 3 (FA 16 ppt, and 33°C) survived for 154 days and no offspring were observed. The individuals in low salinity and low temperature, System 4, (FA 18 ppt and 17ºC) survived for 149 days and no offspring were observed. (Figure13B) *B. exustus* individuals in the control system were alive more than 180 days beyond the termination of the experimental phase and have reproduced effectively.

*Bulla striata: B. striata* is often seen live and is abundant as pristine shell debris from the marginal areas (regions near the Everglades/ Florida Bay interface) of Florida Bay. The experimental results confirm the resilience of *B. striata* to a broad range of salinity and temperature values. *B. striata* individuals survived for 180 days in increasing salinity and increasing temperature System 1, (FA 58 ppt and 31°C), with juveniles observed in the system on 2/18/2004. The individuals in the increasing salinity and decreasing temperature System 2 (FA 58ppt and17.51°C) survived for 175 days with juveniles recorded on 12/22/2003 (Figure 14A). The hyposaline higher temperature individuals System 3, (FA 19 ppt and 33°C) were alive for 131 days with no juveniles seen. The individuals in the hyposaline, low temperature System 4, (FA 16 ppt and 18°C) survived for 159 days with no juveniles found (Figure 14B). Seventy percent of the original individuals were alive in the control system at the termination of the experiment. Reproduction in the control system was observed in large numbers. *B. striata* was resilient across the experimental range and had an abundance of juveniles



### **Table 2. Duration data for the experimental systems. Survival is in days for individual species in each of the experimental systems**







Figure 13A. *Brachidontes exustus* % remaining alive within the experimental systems.





Figure 13B. *Brachidontes exustus* % remaining alive within the experimental systems.





Figure 14A. *Bulla striata* % remaining alive within the experimental systems.







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Figure 14B. *Bulla striata* % remaining alive within the experimental systems.

appearing in the hyper-salinity tanks within 150 days. Salinities when the juveniles were first seen were 45-50 ppt, and these new individuals remain in the systems one year later.

*Cerithium* **sp. cf.** *C. eburneum***:** *C.* sp. cf. *C. eburneum* is more commonly found in the outer estuarine to marine waters where salinity and temperature extremes are less frequent. As expected, this species had a relatively low tolerance for changes in salinity and temperature. All of the original test individuals were dead within 149 days in the hypersaline, high temperature System 1 (FA 50 ppt and 28°C) with no reproduction observed. The individuals in hypersaline low temperature System 2 (FA 38 ppt and 18°C) survived for 120 days and no offspring were observed (Figure 15A). The hyposaline high temperature System 3 (FA 25 ppt and 28°C) individuals survived for 96 days with no offspring seen. The individuals in the hyposaline, decreasing temperature System 4 (FA 25 ppt and 18°C) survived 92 days with no juveniles observed (Figure 15B). The control system also lost most of the original individuals (possibly due to a temperature spike causing stress on 12/22/03. There were, however, juveniles seen in the control system on 01/14/2004 and 3/17/2004. The offspring are still present and growing one year later.

*Cerithium muscarum: C. muscarum* proved to be very resilient in all trial scenarios. The individuals in the hypersaline, high temperature System 1 (FA 62 ppt, and 34°C), survived for 200 days, and juveniles were observed throughout the experimental system by 1/30/2004. The juveniles remain in the system one year later and are reproducing as well. The individuals that were placed in the hypersaline, low temperature System 2 (FA 57 ppt, and 17°C) survived for 172 days, and juveniles were observed in the system on 1/26/2004 and remain in the system one year later (Figure 16A). The hyposaline, high temperature System 3 (FA 17 ppt, and 33°C) individuals survived for 152 days and no juveniles were observed. The individuals placed in the hyposaline, low temperature System 4 (FA 16 ppt, and 16°C) survived for 159 days with no reproduction observed (Figure 16B). Sixty five percent of the original individuals remained alive in the control system at the termination of the experiment. *C. muscarum* has been observed in a wide range of salinities and temperatures *in situ* so these results were not unexpected; however, the proliferation and resilience of the offspring throughout all of the tanks within a system, including the refugia was unexpected.

*Chione cancellata: C. cancellata* is found commonly throughout Florida Bay and surrounding marine waters. These experiments were in large part designed to determine the range of salinity tolerance of *C. cancellata*. The individuals in the hypersaline, high temperature System 1 (FA 57 ppt and 31°C) survived for 161 days and no juveniles were seen. The hypersaline low temperature System 2 (FA 53 ppt and  $16^{\circ}$ C) individuals were alive for 142 days with no offspring found (Figure 17A). The individuals in the hyposaline high temperature System 3 (FA 19 ppt and 31°C) survived for 139 days and no juveniles were found. The individuals in the hyposaline low temperature System 3 (FA 20 ppt and 15°C) survived for 144 days with no juveniles observed (Figure 17B). Fifty percent of the individuals in the control system, maintained at marine salinities and temperatures, survived beyond the completion of the experimental phase.

*Columbella rusticoides*: *C. rusticoides* is found throughout Florida Bay and into the surrounding obligate marine regions. While the number of individuals observed *in situ* is limited, this species has been observed in a range of salinities and temperatures. The individuals in the hypersaline, high temperature System 1 (FA 61 ppt, and  $32^{\circ}$ C)



Figure 15A. *Cerithium sp. cf. C. eburneum* % remaining alive within the experimental systems.









Figure 16A. *Cerithium muscarum* % remaining alive within the experimental systems.





Figure 16B. *Cerithium muscarum* % remaining alive within the experimental systems.





Figure 17A . *Chione cancellata* % remaining alive within the experimental systems.





Figure 17B. *Chione cancellata* % remaining alive within the experimental systems.

survived for 186 days with no juveniles found. The hypersaline, low temperature System 2 (FA 53 ppt, and 16°C) individuals survived for 141 days with no reproduction observed (Figure 18A). The individuals placed in the hyposaline, high temperature System 3 (FA 23 ppt, and 33°C) survived for 124 days with no juveniles found. The hyposaline, low temperature System 4 (FA 24 ppt, and 15°C) individuals survived for 89 days with no reproduction observed (Figure 18B). Thirty percent of the control system individuals survived beyond the close of the experimental phase, and a small group of juveniles were observed.

*Modulus modulus*: *M. modulus* is commonly found throughout Florida Bay and in widely varying salinities and temperatures. The hypersaline, high temperature System 1 (FA 61 ppt, and 32°C) individuals survived for 190 days but no offspring were observed. The hypersaline, low temperature System 2 (FA 57 ppt, and 17°C) individuals survived for 172 days and juveniles were observed throughout the entire system including the refugium tank on 2/18/2004 (Figure 19A). The individuals in the hyposaline, high temperature System 3 (FA 17 ppt, and 32°C) survived for 152 days with no juveniles observed. The hyposaline, low temperature System 4 (FA 21 ppt, and 15°C) individuals survived for 141 days with no juveniles observed (Figure 19B). Fully sixty percent of the original individuals in the control system were alive at the end of the experimental phase, and of these fifty percent were alive over one year later. Juveniles have spread throughout the entire system and have been utilized to begin other experiments.

*Prunum apicinum*: *P. apicinum* is a common inhabitant of seagrass beds throughout Florida Bay and Biscayne Bay, and has been observed in a range of salinities 25-45 ppt. The individuals in the hypersaline and high temperature System 1 (FA 60 ppt and 33 º C) survived for 191 days and no offspring were observed. The individuals in the hypersaline, low temperature System,( FA 49 ppt, and 24ºC) survived for 87 days with no reproduction observed (Figure 20A). The individuals in the hyposaline, high temperature System 3 (FA 20 ppt and 33 º C) survived for 131 days with no reproduction observed. The hyposaline, low temperature System 4 (FA 28 ppt and 23ºC) individuals survived for only 57 days (Figure 20B). Three out of five individuals in the control system survived beyond the termination of the experimental phase but reproduction was not observed. The die-off of *P. apicinum* in this experiment may be more due to lack of proper food source than to the salinity/ temperature levels at the time of demise.

*Turbo castanea*: *T. castanea* is widely distributed in Florida Bay and in obligate marine waters throughout South Florida. It is a good indicator of stable average salinities and provides a euhaline comparison to the overall experimental results. The individuals in the hypersaline, high temperature System 1 (FA 51 ppt and 29°C) survived for 124 days, with no observed offspring. The individuals in the hypersaline, low temperature System 2 (FA 47 ppt, and 23°C) survived for 93 days with no reproduction observed (Figure 21A). The individuals placed in the hyposaline, and high temperature System 3 (FA 24 ppt, and 34°C) survived for 122 days with no observed reproduction. The hyposaline, low temperature System 4 (FA 27 ppt, and 20<sup>o</sup>C) individuals survived for 64 days with no juveniles observed (Figure 21B). The control system had a survival rate of nine out of the original ten individuals and reproduction in very high numbers.

Examining the results for all the species in the four experiments, it appears that salinities higher than 35 ppt (typical marine) and below 45 ppt have a minimal overall effect on the nine species tested (experiments 1 and 2). When the salinity values





Figure 18A. *Collumbella rusticoides* % remaining alive within the experimental systems.







Figure 19A. *Modulus modulus* % remaining alive within the experimental systems.





Figure 19B. *Modulus modulus* % remaining alive within the experimental systems.





Figure 20A. *Prunum apicinum % re*maining alive within the experimental systems.





Figure 20B. Prunum apicinum % remaining alive within the experimental systems.





Figure 21A. Turbo castanea % remaining alive within the experimental systems.





Figure 21B. Turbo castanea % remaining alive within the experimental systems.

exceeded the 45 ppt range, however, mortality rates began to increase. Conversely, in the lower salinity experiments (3 and 4) a decrease of just 9 ppt from 35 ppt to 26 ppt had an immediate effect on the mortality rate in almost all species tested. In contrast to salinity, temperature appears to be a secondary stressor in all of the experimental scenarios. In experiment 3 and 4 the temperature oscillations within the experimental systems did not exceed typical conditions in Florida Bay when the die-off of multiple species began. However, rapid temperature changes seen in experimental mesocosms 1 and 2 on 12/27/2003 in System 1 and 12/29/2003 in System 2 clearly added a level of stress. The die-off of several individuals in both Systems 1 and 2 following the rapid temperature changes demonstrates the effect that wide range-short duration temperature changes have on these species. (Figure 22 of all graphs). These temperature fluctuations are also observed *in situ* and clearly need further experimentation in order to quantify slow stressors coupled with rapid change stress.

#### **DISCUSSION**

Almost all ecosystems have a built in resilience and can tolerate change whether it is a rapid change of short duration or a more gradual change that is sustained for long periods of time. Rapid change will stress a system, sometimes bringing dramatic declines in populations, but generally there are enough survivors to re-establish a stressed species, as long as the duration of the event is short. Changes over longer, biologically significant periods of time, allow an ecosystem to adapt and new or more resilient organisms can fill empty niches. The ecosystem in South Florida and in particular Florida Bay has shifted away from its natural patterns for the past 50-100 years due to changes in freshwater influx. The construction of canals has removed the natural seasonal cycles of freshwater sheet flow from the terrestrial Everglades and has led to point-source delivery of freshwater. This point source (canal mouth) delivery can cause rapid changes in salinity, temperature, and other water quality parameters. Rapid changes that are maintained for extended periods of time do not allow the organisms to adapt or to migrate and large dieoffs can occur, which in turn lead to significant changes in water chemistry as nutrients from decaying organic matter are released. These changes can create a negative feedback loop as added nutrients can cause additional die-offs.

The experimental results presented here have demonstrated that changes in salinity and/or temperature can cause polyhaline organisms to be stressed beyond the limits of recovery, for example in the decreasing salinity systems no reproduction was observed in any system. This loss will inevitably lead to a change in the community composition that is found within the affected areas, beginning with a reduction in overall diversity especially in environments where only the resilient euryhaline species can survive. Our field observations (unpublished data:

http://sofia.usgs.gov/exchange/flaecohist/) indicate this loss of diversity has already happened in the vicinity of Taylor Creek and Trout Creek outflow where we find a

predominance of *Brachidontes exustus* and *Cerithium muscarum*. When the plant and invertebrate populations are stressed beyond recovery, the overall effect can be farreaching as these organisms form the base of the food chain for most of the other inhabitants of these regions. These estuaries also serve as large nurseries for many marine fish and invertebrate species.

By combining data from the LSC experiments and from ongoing Ecosystem history research, molluscan species can be identified as indicator species to measure the overall health of South Florida estuaries, particularly Florida Bay. Indicator species of minor or short term changes that exceed seasonal variation and are commonly found throughout the Florida Bay region include *Modulus modulus* and *Brachidontes exustus*. These two species are relatively abundant and easily found alive and in debris samples, but as the experiments have shown, have a lower tolerance to water changes just beyond the typical range of seasonal variation. Indicators of sustained, large scale changes include *Cerithium muscarum*, and *Bulla striata*. These two species appear to have a strong resilience during changes in salinity and temperature beyond the typical range of seasonal variation, but beyond which survival is extremely limited. These observations are confirmed by our modern field observations and collections from Florida Bay, taken since 1995 (unpublished data http://sofia.usgs.gov/exchange/flaecohist/) The offspring produced by both *C. muscarum* and *B. striata* were able to survive for more than three months in the higher salinities and began to show rapid growth when the experimental tanks were slowly returned to typical seasonal ranges of salinity and temperature.

The emphasis of the experiments was on salinity and temperature; however, we observed the impact of other factors on the study species. *Prunum apicinum* and *Columbella rusticoides* have more specific food source needs than the epiphytic grazing species in this experiment and did not survive throughout the range of salinities that have been observed *in situ*. Subsequent observations have shown that *P. apicinum* will prey on smaller gastropods and is a very aggressive carrion feeder with response time to food source introduction measured in seconds to minutes over the total tank area. When provided with adequate food sources *P. apicinum* will survive and flourish in our systems. The food source supply problems also may have affected *Brachidontes exustus*. *B.exustus* that were introduced into System B have shown good resilience and reproduction in part due to overfeeding designed to stimulate an increase in nutrients. Subsequent observations show that *B. exustus* will thrive when a range of phyto-plankton, pico-plankton and large algal mats, primarily Phaeophytes and filamentous Chlorophytes are present in the system. These observations have been seen both *in situ* and in our mesocosms. Eutrophication of the water column will often lead to pico/phyto plankton blooms that may explain the dispersal of *B. exustus* in the Bay waters. *Turbo castanea,* being obligate marine, survived well in the control system but did not survive well in any experimental scenarios.

*Chione cancellata* proved to be a relatively resilient species across the range of experiments as expected because it is observed alive throughout Florida Bay. This experimental phase was used to define the overall range of tolerance and to begin to quantify the outside limits of the salinity and temperature range for *C. cancellata*. These data will be coupled with the ongoing biogeochemistry data that we are accumulating for the biogeochemistry studies on *Chione*.

In contrast to *Chione cancellata*, observations of living *Cerithium* sp. cf. *C. eburneum* and *Turbo castanea* have been restricted to marine to polyhaline environments in the regions in and around Florida Bay. *C.* sp. cf. *C. eburneum* demonstrated a narrow range of tolerance to salinity and temperature changes in all experiments, which agrees with our *in situ* observations in Florida Bay. *Turbo castanea* survived well in the control system but did not survive well in any experimental scenarios.

The nine species in this experiment represent common members of the overall assemblage of mollusks in Florida Bay. By the very nature of the experimental process however, the tested organisms are biased towards: 1) species that can survive the rigors and stress of relocation and equilibration into the mesocosms at LSC; and 2) species that are relatively abundant and easy to locate and collect in the field. Given this sampling bias, we expected the selected species to be tolerant of a range of salinities and temperature within seasonal limits, and the experiments demonstrated that they were. The mortality rates began to change, however, when we exceeded the typical seasonal range taking the systems to recorded highs and lows of salinity and temperature. With few exceptions the broad range (60-0.0 ppt) of temperatures and salinities, which have been recorded in situ (Figure 4), caused a large die-off of the original test organisms. The experimental results demonstrate that even the most common and resilient members of the ecosystem have a point at which survival is very limited.

The South Florida ecosystem has evolved to withstand changes due to extremes in weather, limited nutrient loading and subtle overall changes. However, when seasonal changes are coupled with point source stresses, brought about by controlled water delivery for example, the resulting pressure may be overwhelming. A better understanding of why and how these resilient species cope with changes in salinity and temperature is needed as is experimentation on rapidly changing cycles of salinity and temperature. These data should provide another indicator by which resource mangers can monitor change so that habitat loss can be averted.

#### **SUMMARY**

The primary goal of the Comprehensive Everglades Restoration Project is to restore more natural freshwater flow through the south Florida ecosystem including quantity and timing of freshwater deliveries into the system, and maintaining water quality within the overall system. Understanding the effect of salinity and temperature, beyond typical oscillations, on the biota of the South Florida estuaries is necessary for the successful outcome of the overall goals of CERP. These experiments addressed two aspects of the Ecosystem History Projects: 1) determining the utility of using molluscan shells as recorders of change in water chemistry parameters, primarily salinity, and 2) enhancing our *in situ* observations on modern assemblages by exceeding typically observed conditions. These experiments allowed us to gain an understanding of the effects of salinity, temperature and other water chemistry parameters on the reproduction, growth and overall survivability of key species of mollusks, used in interpreting sediment core data.



Observations on mollusks, plants and microbes made as part of these experiments have further refined our knowledge and understanding of the effects of ecosystem feedback and what role salinity and temperature play in ecosystem stability. Data collected on the effects of salinity and temperature driven changes need to be verified or tested in the field. The mesocosms and experiments were designed in part based on observations made in South Florida estuaries. Observations made in the mesocosms, on a scale not normally observable in the field, have led to new questions about the influence of salinity on the localized ecosystem, which need to be tested *in situ*.

The results have demonstrated the viability of several molluscan species (for example *Cerithium muscarum*, and *Bulla striata* due to the ability to withstand a broad salinity range) that could be utilized as indicators of atypical salinity, and possibly temperature, oscillations. These data and the use of these species can provide a more accurate measure of anthropogenic influence in the South Florida estuaries. The experiments also have provided calibration data for the shell biogeochemistry of *Chione cancellata* and the use of this species as a water chemistry recorder. The next phase of experiments will test the stress levels/survival rates of repeated, short duration changes of salinity and temperature at various intensities. We would also like to expand the biogeochemistry study of *Chione cancellata* to include several of the gastropod species utilized in this experiment.

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# **Species List**

# **Plants:**

# **Algae**

### **Chlorophyta**

*Avrainvillea nigricans Batophora occidentalis var. largoensis Batophora oerstedii Caulerpa paspaloides Caulerpa prolifera Caulerpa cupressoides Caulerpa sertularoides Caulerpa serulata var. hummii Caulerpa ashmeadii Caulerpa mexicana Caulerpa racemosa var. occidentalis Caulerpa racemosa var. peltata Caulerpa taxifolia Chaetomorpha crassa Chaetomorpha linum Cladophora liniformis Cladophora prolifera Dictyosphera cavernosa Dasycladus vermicularis Neomeris annulata Enteromorpha compressa Enteromorpha flexuosa Batophora oerstedi Penicillis capitatus Penicillis dumetosus Phipocephalus phoenix Acetabularia crenulata Acetabularia schenkii Anadyomene stellata Halimeda monile Halimeda opuntia Halimeda incrassata Halimeda discoidea Halimeda tuna* 

*Udotea flabellum Udotea cyathiformis Valonia ventricosa Valonia cavernosa Valonia aegagropila Valonia macrophysa Valonia utriularis* 

### **Rhodophyta**

*Polysiphonia subtillisima Spyridia filimentosa Laurencia sp. Digenia simplex Hydrolithion farinosum Pneophyllum fragile Titanoderma pustulatum Hypnea musciformis Gracilariopsis lamaneiformis Lomentaria baileyana Ceramium brevizonatum var. caraibicum Tiffaiella gorgonea Dasya harveyi Dasya ocellata Dasya ramosissima Heterosiphonia crispella var. laxa Chondria atropurpurea Polysiphonia havanensis Polysiphonia opaca Wrightiella tumanowiczii* 

### **Phaeophyta Order Dictyotales**

### **Family Dictyotaceae**

*Dictyota cervicornis Dictyota dichotoma Dictyota crispate Dictyota crenulata Dictyota mertensii*  **Order Ectocarpales Family Ectocarpaceae**  *Feldmannia indica* 

## **Order Scytosiphonales Family Scytosiphonaceae**

*Rosenvingia intricate* 

## **Order Fucales Family Sargassaceae**

*Sagassum filpendula Sargassum natans Sargassum fluitans* 

**Angiosperms** 

*Thalassia testudinum Halodule wrightii Syringodium filiforme Rhizophora mangle Ruppia maritima* 

## **Mollusks**

*Prunum apicinum Cerithium musacarum Cerithium eburneum Cerithium sp. Modulus modulus Modulus sp. Astraea americana Astraea phoebium Tricolia affinis Nerita sp. Rissoina sp. Caecum pulchellum Caecum sp. Vermicularia sp (3) Batillaria sp. Crepidula sp (2) Strombus gallus Mitrella sp. Mitrella sp. Bulla striata. Conus sp. Tegula sp. Corphella verrucosa (nudibranch)*  **Order:** SACOGLOSSA **Superfamily:** OXYNOOIDEA **Family:** Oxynoidae *Oxynoe viridis Carditamera floridana Columbella rusticoides Columbella sp. Lucina sp. Argopectin sp. Tellina sp. (2+) Chione cancellata Brachidontes exustus Diodora sp. Diodora sp. Laevicardium sp. Pinna sp.* 

*Atrina sp. Noetia sp.Barbatia sp. Arca zebra Arca sp. Chiton tuberculatus Pteria imbricate Pteria colymbus Turbo castanea* 

### **Echinodermata**

Sea urchins (3) sp. Sand dollars

#### **Holothurians**

Sea cucumbers 3+sp.

### **Ophiuroidea**

Brittle stars 6+ sp.

#### **Asteroidea**

Sea stars (3) sp.

### **Bryzoans**

#### **Cnidarians**

#### **14 species of hard and soft corals**

*Condactylus gigantea Aptasia sp.*  As yet unidentified anemone

#### Crustacea

#### **Pagurus acadianus**

**Common Name:** Acadian Hermit Crab **Team:** Crustaceans **Phylum:** Arthropoda

## **Gammarus spp**

**Common Name:** Sand Flea **Team:** Crustaceans **Phylum:** Arthropoda

Mithrax sculptis Lobsters

Peppermint shrimp Arrow crabs

### **Cyanobacteria**

*Hydrocoleum coccineum Dichothrix penicillata*