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## Factors that Affect Distribution of Two Species of Killifish in Northeast Florida Marshes

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Factors that Affect Distribution of Two Species of Killifish in Northeast Florida Marshes

By

Stacy N. Galleher

A thesis submitted to the Department of Biology  
in partial fulfillment of the requirements for the degree of

Master of Science in Biology

UNIVERSITY OF NORTH FLORIDA

COLLEGE OF ARTS AND SCIENCES

June 2007

CERTIFICATE OF APPROVAL PAGE

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## Abstract

*Fundulus heteroclitus* and the closely related *F. grandis* are mainly distributed along the Atlantic coast of the U.S from Maine to Northeast Florida and from the East coast of Florida throughout the Gulf of Mexico, respectively. Both are resident salt marsh fishes whose range is thought to overlap in Northeast Florida, making them an ideal study system to examine resource partitioning between two closely related species. The objective of this study was to examine the effects of temperature and elevation on potential habitat partitioning of these two species. It is hypothesized that the northern species, *F. heteroclitus*, would have a lower thermal tolerance than *F. grandis* and would be found in lower marsh elevations, which are thought to be slightly cooler. *Fundulus heteroclitus* larval and juvenile distribution was examined and elevation was found to be significant ( $p < 0.001$ ) in the distribution with smaller fish utilizing higher elevation areas. Temperature was not found to differ between elevation sites, thus could not account for elevational differences. To further determine the role of temperature in *Fundulus* distribution, both species were hatched in the laboratory, and larvae were used for critical thermal maxima trials. *F. heteroclitus* from one population had significantly higher mean loss of equilibrium (LOE) temperatures ( $p < 0.003$ ) than either *F. grandis* populations. Due to population differences results could not be pooled by species. Critical thermal maximum temperatures show that both species can tolerate roughly the same extreme high temperatures and, based on temperature alone, should be able to live in the same habitat. Temperature tolerance of both species was higher than the actual measured field temperatures and is therefore not a likely factor in determining both species' range. A



combination of other abiotic factors and biotic interactions such as competition may play a greater role in determining the observed range of each species than previously thought.

## Chapter 1 General Introduction

### *General Resource Partitioning*

Organisms are adapted to inhabit only a small range of biological parameters that exist. Within those parameters, abiotic and/or biotic interactions limit how much tolerable range the species actually occupies. An actual niche encompasses the outer extreme limits a species can physiologically tolerate. Due to abiotic factor(s) and biotic interactions, organisms often occupy only a portion of their actual niche, known as their realized niche. Patterns of large scale distribution may be determined by examining what factors confine a species to its realized niche. Closely related species often have overlapping resource needs and may be susceptible to intense competition or other methods of niche separation under varying conditions. Resource partitioning, or partial utilization of a resource between species with overlapping resource needs, can be useful in identifying the boundaries of a realized niche and the interactions between species. Studying resource partitioning may help determine the interactions that influence a species' realized niche and the limitations of its distribution.

Resource partitioning can take many different forms in fish. For example, different feeding behaviors or selective feeding is an example of a type of biotic resource partitioning. Plastic foraging methods, such as a shift from benthic to drift foraging [Nakano et al., 1999 (charr)]; size selective prey use [Gladfelter and Johnson, 1983(squirrel fish); Wynes and Wissing, 1982 (darters)]; territory and resource protection

[Robertson, 1984; Ebersole, 1985 (damsel fish)] are examples of different feeding methods that can determine niche breadth. Competition is often a key factor in most biotic interactions that result in niche partitioning into different microhabitats, where each species has less overall niche overlap.

### *Effects of Abiotic Factors*

Abiotic characteristics have been considered as background limiting factors that determine species ability to participate in biological interactions. Those biological interactions, often some form of competition, are what ultimately control species distribution, not any confounding abiotic factors (Dunson and Travis, 1991). Although, abiotic factors direct which species are within close proximity to induce biological interactions, they do not necessarily make all competitors equal. By definition species must be able to tolerate all physiological conditions of their habitat. However, one species may be slightly better suited to a specific group of abiotic factors than another. If abiotic conditions change, one competitor could be favored over the other (Hutchison, 1961). Therefore, any further biotic interactions will be a result of both the abiotic limitations that provided the initial pool of species and the interactions altered by the fluctuating abiotic conditions.

Abiotic conditions can influence the results of competition and play a role in habitat partitioning and distribution. An example of abiotic factors that partition resources is low salinity causing low growth and reproduction in Sheepshead Minnow, *Cypridodon variegatus*, limiting its distribution in freshwater habitats (Dunson et al., 1998). Another example is salinity tolerances affecting the outcome of competition for food between the killifish, *Lucania parva* and *L. goodei*. *Lucania parva* is favored in

15ppt water and *L. goodiei* is favored in fresh hard water (Dunson and Travis, 1991). Interspecific competition between *Lucania spp.* prevents either species from dispersing into nearby habitat occupied by conspecifics even though both species could physiologically tolerate the other fundamental niche. Also, salt marsh plants from New England and possibly across the East coast were found to have different competitive interactions under different levels of nutrient and tidal stress, where dominance shifted between stress tolerant plants and non-stress tolerant plants (Emery et al. 2001; Pennings et al. 2002). Eurytolerant organisms may show differences in competitive ability in harsh changing habitats that are often occupied by fewer species. Also, energy efficient responses may make eurytolerant organisms better competitors than organisms from more stable environments. Previous literature has shown that it is possible to model distributions of species by understanding the interactions between the abiotic and biotic characteristics of their habitat (Magnuson et al., 1979).

### *Model Organism*

Fundulidae is a family of Cyprinodontiformes fish commonly called topminnows and killifishes which contains species that inhabit fresh to hypersaline waters. This family of fish is commonly used in laboratory studies ranging from embryology, decapsulating or dechorionating eggs; genetics work with emphasis on genetic variation; endocrinology, specifically looking at pigmentation of chromatophores; to toxicology, effects of various chemicals on all aspects of life history (reviewed by Atz, 1986 and Powers et al., 1986). A majority of these studies have been performed on the Common killifish, *Fundulus heteroclitus* (Linnaeus 1766), commonly called the aquatic equivalent to the laboratory rat (Atz, 1986).

*Fundulus heteroclitus* and the closely related Gulf killifish, *F. grandis* (Girard, 1859), are mainly distributed along the Atlantic coast of the U.S from Newfoundland to Northeast Florida and from the East coast of Florida throughout the Gulf of Mexico, respectively. Adults predominantly reside in tidal creeks and access the marsh surface during high tide to feed and reproduce (Kneib, 1986; DiMichelle and Taylor 1980). Both *F. heteroclitus* and *F. grandis* are unique resident salt marsh fish that spend their entire larval developmental period and all other stages of their life history in the harsh tidal dependent habitat of the marsh surface (Lipecius and Subrahmanyam 1985; Kneib 1986; Weisberg 1986). Because it is a commonly studied laboratory organism and much is already known about its physiology, ecology, and genetics, *F. heteroclitus* and *F. grandis* are an ideal study organism.

#### *Objectives*

The overall objectives of this study are to (1) determine the distribution and abundance of larvae and juvenile *F. heteroclitus* on the marsh surface at the Southern end of its range; (2) determine if abiotic factors affect population characteristics and (3) to determine if differences in abiotic tolerances exist between the closely related *F. heteroclitus* and *F. grandis* and whether they govern the distribution pattern found in nature.

## Chapter 2 Larvae and juvenile *Fundulus heteroclitus* abundance and distribution in Northeast Florida Marshes

### *Introduction*

*Fundulus* play an important role in salt marsh energetics, as a predator and a source of food at all stages of life history for fish and other commercially important invertebrates (Kneib, 1986; Allen et al., 1994). Although *Fundulus* are nearly ubiquitous in salt marshes along the East coast, the marsh habitat is not a homogenous environment and patchy distributions of organisms exist (Rozas, 1995). Also, patchy distributions of invertebrates on the marsh surface are well known and have been thought to correspond to direct and indirect effects of *Fundulus* predation (Kneib 1986, 1988). Examining what factors define patchy marsh use by *Fundulus* could be helpful in determining the specific characteristics of a productive marsh. Modeling a set of characteristics, biotic and abiotic, is useful in categorizing areas of protection or delineating areas of interest for marsh restoration. Since *F. heteroclitus* is not very sensitive to minute changes and is a model study organism with much already known, it could be used as an indicator species of large scale problems with marsh health (Atz, 1986).

Few teleosts, all within the genus *Fundulus*, utilize the marsh surface for reproduction due to the extreme abiotic conditions. *Fundulus heteroclitus* have many characteristics, such as egg morphology, that are specifically adapted for development on the marsh surface in shallow marsh depressions. Chorionic papillae and pebble-surfaced filaments attached directly to the chorion are present in different densities and diameters

on *F. heteroclitus* eggs from New Jersey to Florida (Morin and Able, 1983). These papillae and filaments are “sticky” and attach eggs to the base of *Spartina alterniflora* stems and empty mussel shells (*Geukensia demissa*; Taylor and DiMichele, 1983; Able and Castagna, 1975). Adults deposit eggs during spring high tides throughout the reproductive season (April- September) and eggs incubate exposed to the air until the following spring tide. Although eggs have varying development times, on average ~9 days at 20°C, they are often delayed in hatching until the following spring tide which lowers levels of oxygen during inundation (DiMichele and Taylor, 1980; Taylor and DiMichele, 1983). By delaying hatching until the spring tide, juveniles are able to access more marsh depressions and foraging areas than during neap tide. Juvenile size classification is determined by absorption of the yolk sack, roughly 3 days post spawning, and up to a standard length (SL) of approximately 10-13.5 mm (Talbot and Able 1984; Kneib, 1986). Towards the end of the juvenile stage fish begin to move on and off the marsh surface with tidal flow in a similar pattern as adults. Little is known about distribution of *F. heteroclitus* especially during the larval and early juvenile phase. The role of *F. heteroclitus* in marsh energetics will not fully be understood until studies demonstrate the interactions between larvae and juveniles that determine the distribution on the marsh surface (Kneib, 1986).

Abiotic factors play a key role in fish distribution on the marsh surface where conditions can be greatly affected by small scale changes in air temperature and weather. Examining a single abiotic factor is a necessary first step in elucidating the role, if any, of that factor in the distribution of an organism (Sylvester, 1975). Because a multitude of abiotic factors could contribute to the abundance of juveniles, and Fundulids exhibit a

high tolerance to many of these factors such as dissolved oxygen, salinity, pH etc., field studies will be limited to specifically monitoring tidal inundation and temperature.

Previous studies in Sapelo Island, GA have shown differences in marsh use by larval and juvenile *F. heteroclitus* based on elevation changes, with smaller fish inhabiting high marsh areas and larger fish residing close to the marsh edge often in a creek (Kneib, 1986). Little previous information exists about distribution patterns of *F. heteroclitus* in the southern most area of its range, roughly found to be around Jacksonville, FL in Duval and St. Johns counties. However, it is hypothesized that *F. heteroclitus* should display similar distribution patterns as Georgia populations, but may not display such strong correlation to elevation, since changes in elevation are less drastic in Florida. Field temperature effects have not been studied extensively but have been shown experimentally to affect larval and juvenile development (Middaugh et al., 1978; DiMichele and Westerman, 1997; Tay and Garside, 1975). Water temperatures on the marsh surface are not well recorded, especially in relation to fish abundance. Little is known about temperatures experienced on the marsh surface due to the complex nature in studying the many influencing factors. High marsh sites, inhabited by smaller sized fish should show greater thermal variation and extremes due to surface radiation.

#### *Objective*

Determine small scale distribution and abundance of *F. heteroclitus* larvae and juveniles in Northeast Florida marshes in relation to elevation or amount of flooding and changing abiotic characteristics specifically temperature.



## *Methods*

Distribution and abundance of larvae and juvenile *Fundulus heteroclitus* was determined by a series of pit traps in three locations in Northeast Florida. A northern location, Nassau River (30.5209850, -81.4986218), and two southern locations Crescent Beach (29.9507020, -81.3109463) and Pellicer Creek (29.6797363, -81.2245700) are shown in Figure 1. The Nassau site was chosen based on the greatest adult abundances recorded by the Jacksonville field lab of Fish and Wildlife Research Institute (FWRI) – Fisheries Independent Monitoring Program (FIM) from 2001–2004 (Figure 2). All other sites were chosen for similar habitat to the Nassau site to reduce variability between locations. Sites were all situated in a small creek off of the main channel, where less boat traffic may occur. All vegetation at locations and sites was composed of predominantly smooth cord grass, *Spartina alterniflora* with Black needle rush, *Juncus roemerianus* near by but not located within sites. All sites had approximately the same density of grass but no measurements of vegetation or sediment were recorded. At each site, two sets of 3x3 grids with a total of 18 traps (10 cm diameter x 5 cm height pyrex containers) were dug into the marsh and held flush to the surface by 3 metal stakes. Traps were located near the spring high tide mark but close enough to a marsh creek or small rivulet to be completely inundated during spring high tide. A tide marker, a wooden dowel with a float and plastic mesh marker, was placed at each of the four corners of a grid to quantify the maximum inundation per tide cycle per grid. Grids were classified by a “low” and “higher” elevation within each location, characterized by a mean tidal inundation of 45.7 ( $\pm 12.97$ ) cm and 28.7 ( $\pm 13.22$ ) cm, respectively, with the highest and lowest values for maximum inundation recorded for each spring tidal cycle sampling

event (Table 1). Grids were within roughly 30 meters of each other without overlapping in the path of tidal flood. Traps were sampled a minimum of one time after each spring tide from March-July in 2005 for Nassau and Crescent sites and March-July 2006 for all sites. All fish present in traps were transported on ice back to the lab for enumeration and standard length measurements. At the time of each collection air temperature and water temperature for the creek and trap was measured using a Model 85 YSI for each location. Continuous temperature data was recorded from Sept. 30 – Nov. 21, 2006 for the Pellicer Creek location. Continuous trap water temperatures were measured every 15 min from a pit trap located at the center of each grid using an ibutton temperature sensor and digital data logger (model number DS1992L-F50). The ibutton was suspended from a plastic mesh covering the pit to reduce the effects of sediment contaminating the temperature readings. Continuous tide, water and air temperature data were collected from an established data sonde located as close to the study site as possible to cross-reference data and examine trends in temperature data recorded from the traps. Pellicer Creek was the only site used to determine continuous temperature data due to a lack of permanent data sondes and technical difficulties.

### *Statistical Analysis*

Arithmetic means of fish standard length were calculated and a reciprocal transformation was performed to standardize variances among sites and locations. After transformation, length distributions were compared within locations, using a non-parametric Mann-Whitney rank sum tests due to non-normal distributions of lengths. ANOVA and post hoc Tukey's HSD were run to compare mean standard lengths of larvae and juveniles from high and low elevations sites between locations.

Figure 1 – Larval and juvenile pit trap sampling locations in Northeast Florida. Each location contains both high and low elevation sampling grid.

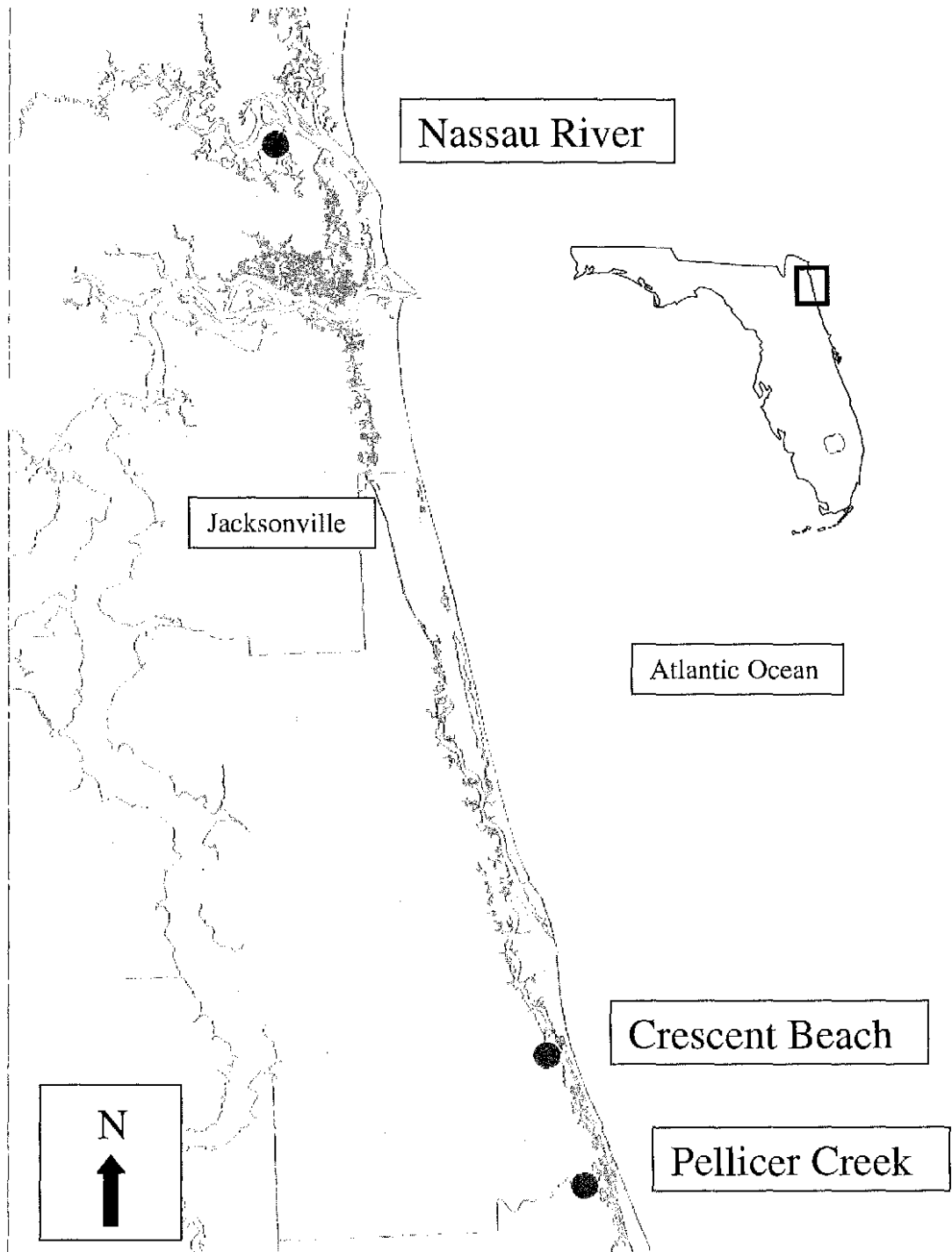


Figure 2 – Adult *F. heteroclitus* abundance collected from Nassau River by Fisheries Independent Monitoring Program (FIM). Total catch (number of fish) was recorded per each seine (21.3 m seine with 3.2 mm mesh set from a boat) sampling event (n=90) using standard FIM protocol from 2001-2004.

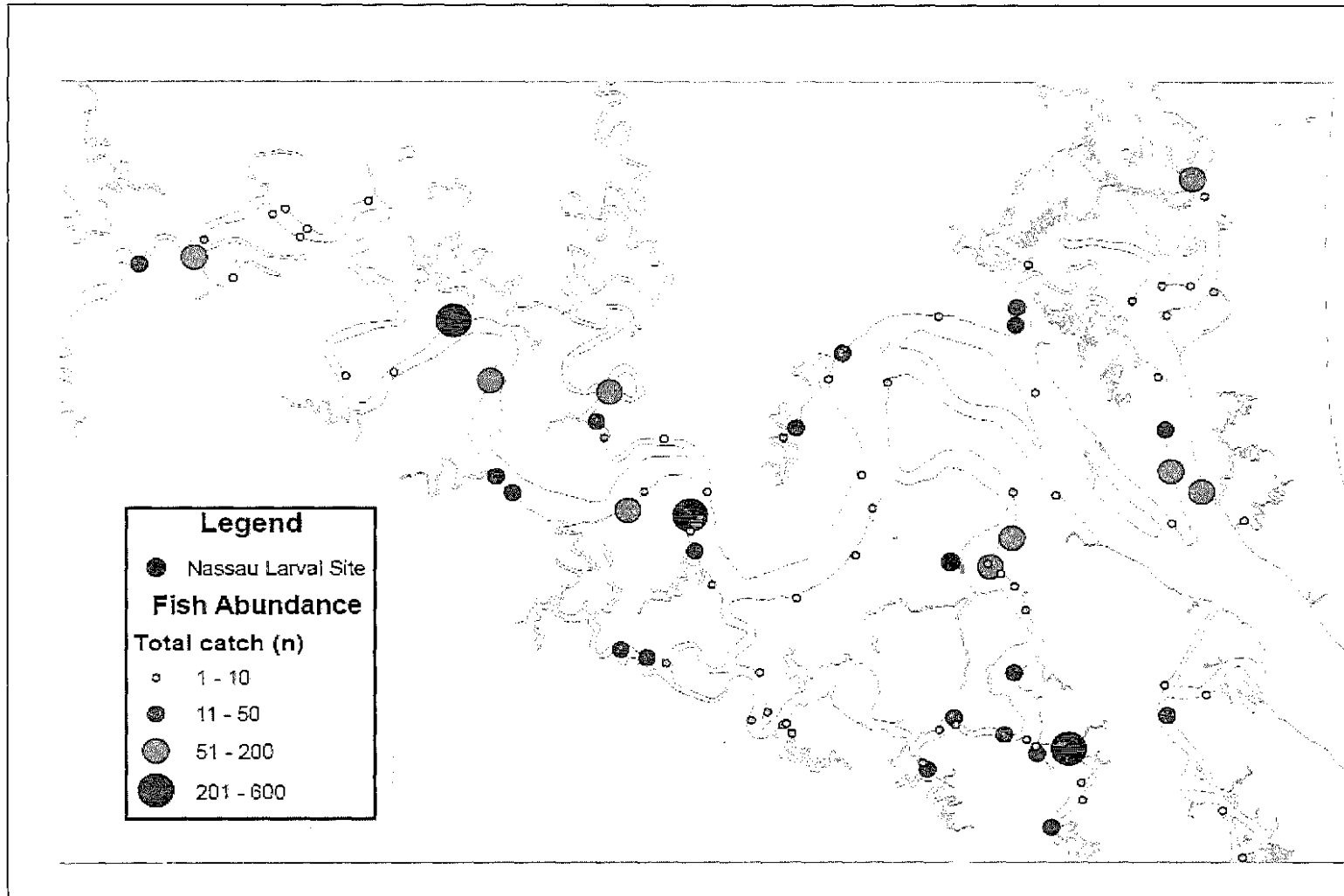


Table 1 – Average maximum tidal inundation for all sites (cm) with highest and lowest maximum tidal inundation for each spring tide cycle sampling event from March – July 2006

	<b>Sites</b>	<b>Mean</b>	<b>Sd</b>
<b>Low</b>	Nassau	44.70	14.15
	Crescent	47.81	14.05
	Pellicer Creek	44.46	10.72
<b>High</b>	Nassau	29.54	12.58
	Crescent	35.55	16.43
	Pellicer Creek	21.12	10.64

## *Results*

### *Larval distribution*

Multiple Mann-Whitney rank sum tests were performed to determine differences in length distribution of fishes from sample sites at different elevations within study locations over two sampled years. Nassau and Crescent fish length distributions were found to be significantly different with a lower mean standard length, at higher elevations than lower elevations for both years ( $p < 0.001$  for all; Table 2). Length distributions from the Pellicer Creek site were found to be significantly different with a lower mean standard length ( $p < 0.004$ ) at high elevations compared to the low elevations (Table 2). Nassau and Crescent high and low elevation site data could not be combined between sample years due to unequal variances, even after transformations. Therefore, locations were compared using only 2006 data to include the Pellicer Creek location. High elevation sites for 2006 showed no significant difference in larval/juvenile length distribution between locations ( $p = 0.110$ , Table 3, Figure 3). At low elevation sites, fish length distribution was reciprocal transformed and found to be significantly different ( $p < 0.001$ , Table 4). Post hoc Tukey's HSD showed larval/juvenile length distributions were significantly different from each other ( $p < 0.001$ ) at each location, except for Crescent and Pellicer Creek ( $p = 0.874$ ) (Figure 3). Fish from low elevation sites at Nassau had a larger mean standard length than low elevation populations from either of the other two sites. Although, the high elevation sites are not significantly different they show a similar trend to the low sites, with Nassau fish being slightly longer than either southern population. Fish abundance was found to be roughly two times greater at all lower elevation sites than higher elevation sites (Table 2).

### *Temperature monitoring*

Two sub-samples were used to determine continuous temperature data due to potential sediment contamination falsely increasing the temperature difference between high and low elevation sites (Figure 4). Periods of low sediment interference were days immediately following manually clearing mud from the lower elevation site. Before and after placement in the field, data were recorded to determine a pre and post calibration error of  $\pm 0.1^{\circ}\text{C}$  between ibutton loggers. Equipment error of ibuttons has been reported as  $0.5^{\circ}\text{C}$  by the manufacturer and data error logged prior to field placement ranges from  $0.05\text{-}0.9^{\circ}\text{C}$ . Sub-sample periods, 10/6/07 – 10/12/07 and 11/3/07 – 11/9/07, are displayed with data from ibuttons and from a permanent data sonde located near the mouth of Pellicer Creek (Figure 5 and 6). Both sampling periods show air temperature was more variable, compared to dampened thermal oscillations for water temperature, and may be impacted by mesoscale thermal influences such as cloud cover or wind shifts. These data also show a positive relationship between tide cycle and grid temperature with a tidal peak preceding site temperature peak for both tides per day, although this trend is more pronounced in the afternoon high tide. Site temperatures were consistently higher than surrounding air or water temperatures suggesting surface radiative warming. The first sample period (Figure 5) was over a spring tide which is reflected in the greater tidal oscillation and corresponding distinct peaks for site temperatures. The second sample period (Figure 6) shows lower amplitude in tidal fluctuation, not a spring tide, and site temperature peaks seem to be dampened compared to the first sample period. The second sample period also has less air temperature variation, possibly due to cloud cover. At the highest site temperature peak of the day, for both sampling periods, differences between

the high and low elevation sites were less than or equal to the amount of error associated with the logger (roughly 1 °C). The small amount of variation present in thermal peaks between sites of different elevations is roughly equal and can be attributed to a delay in movement of water across the surface of the marsh, affects of air temperature, or possible partly cloudy conditions. Because differences in temperature for high and low sites over these two periods are not greater than the error associated with the loggers, it can be extrapolated, as long as conditions were similar between the temperature sampling and larvae sampling periods, that there is no overall difference in temperature between sites. If there is no difference in temperature over the sampling period, then temperature alone could not cause the difference in abundance and length distribution presented in this study.



Table 2 – A series of separate Mann-Whitney Rank Sum tests performed on standard length (SL) distributions for all locations for both sampling years. All data reciprocal transformed for homogeneity of variances. Significant comparisons signified in bold.

Location	Year	N	Mean SL (mm)	Mean Rank	Sum of Ranks	Mann-Whitney U	Sig.
Nassau 05	High	152	7.7090	253.48	38529.00	10947.000	<b>0.000</b>
	Low	249	9.3092	168.96	42072.00		
Nassau 06	High	61	9.1285	151.25	9226.50	2180.500	<b>0.000</b>
	Low	156	13.0641	92.48	14426.50		
Crescent 05	High	150	7.5024	276.29	41444.00	18781.000	<b>0.000</b>
	Low	326	8.4407	221.11	72082.00		
Crescent 06	High	50	8.2316	99.79	4989.50	1735.500	<b>0.000</b>
	Low	109	10.9900	70.92	7730.50		
Pellicer Creek	High	5	6.5240	10.30	51.50	36.500	<b>0.004</b>
	Low	66	10.2326	37.95	2504.50		

Figure 3 – Mean larval length distributions from 2006. Significance indicated by letter from reciprocal transformed (for low sites) ANOVA and post-hoc Tukey’s HSD. For low sites, Nassau was significantly different from Crescent and Pellicer Creek ( $p < 0.001$  for both) and Crescent and Pellicer Creek locations were not significantly different ( $p = 0.874$ ).

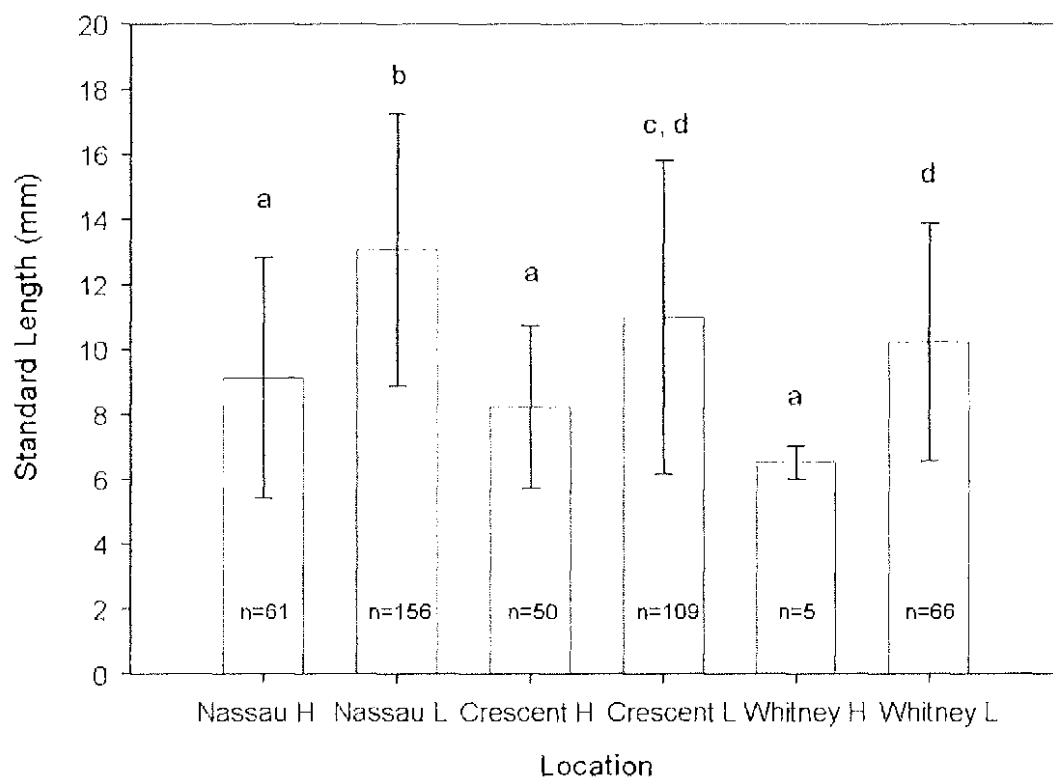


Table 3 – ANOVA of mean length of *F. heteroclitus* from high elevation sites in all locations from 2006.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	45.272(a)	2	22.636	2.253	0.110
Intercept	2413.144	1	2413.144	240.198	0.000
location	45.272	2	22.636	2.253	0.110
Error	1135.253	113	10.046		
Total	9819.155	116			
Corrected Total	1180.525	115			

Table 4 – ANOVA of reciprocal transformed comparison of mean length of fish from low elevation sites in all locations from 2006.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.035(a)	2	0.017	14.487	<b>0.000</b>
Intercept	2.950	1	2.950	2461.606	0.000
Location	0.035	2	0.017	14.487	0.000
Error	0.393	328	0.001		
Total	3.564	331			
Corrected Total	0.428	330			

Figure 4 – Difference in temperature between high and low elevation sites at Pellicer Creek over a secondary test period. Tide data from a permanent data sonde located at the mouth of the creek.

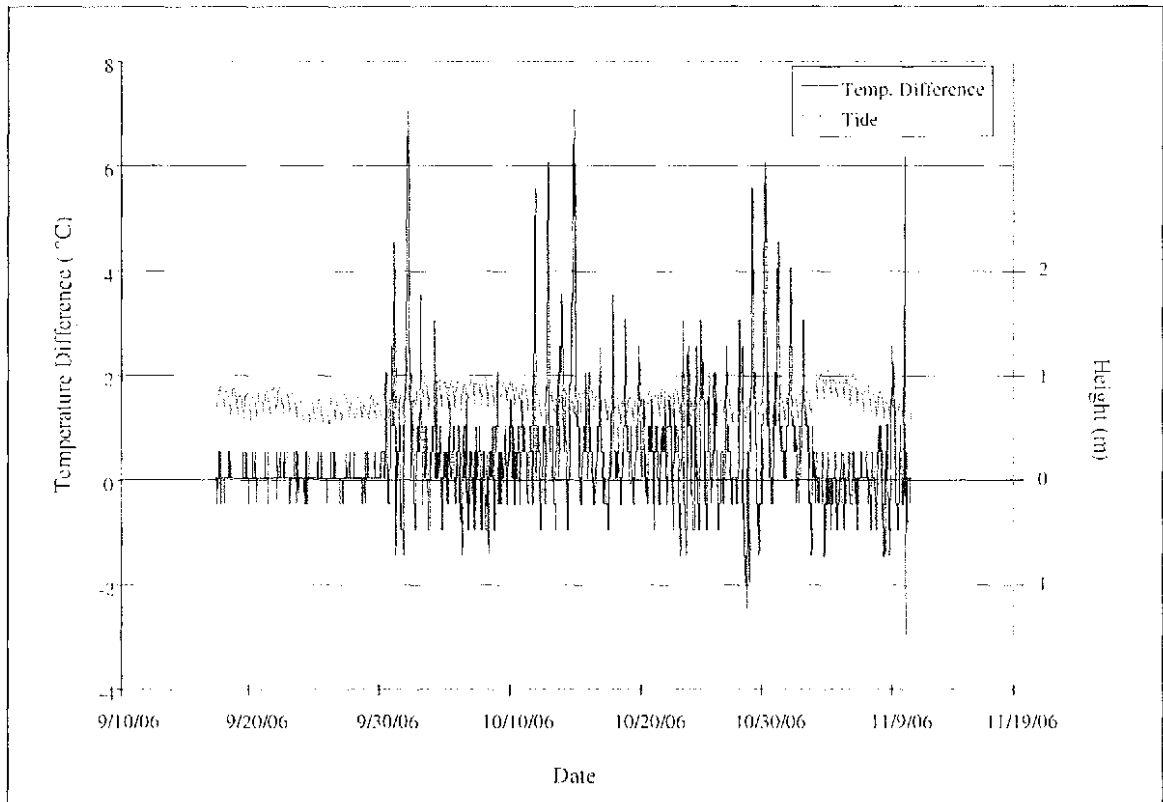


Figure 5 – First test period for Pellicer Creek from October 6-12, 2007. Tide, air and water temperature data from permanent data sonde located at mouth of Pellicer Creek. Pellicer low and high elevation sites located at 29.6797363N, -81.2245700W.

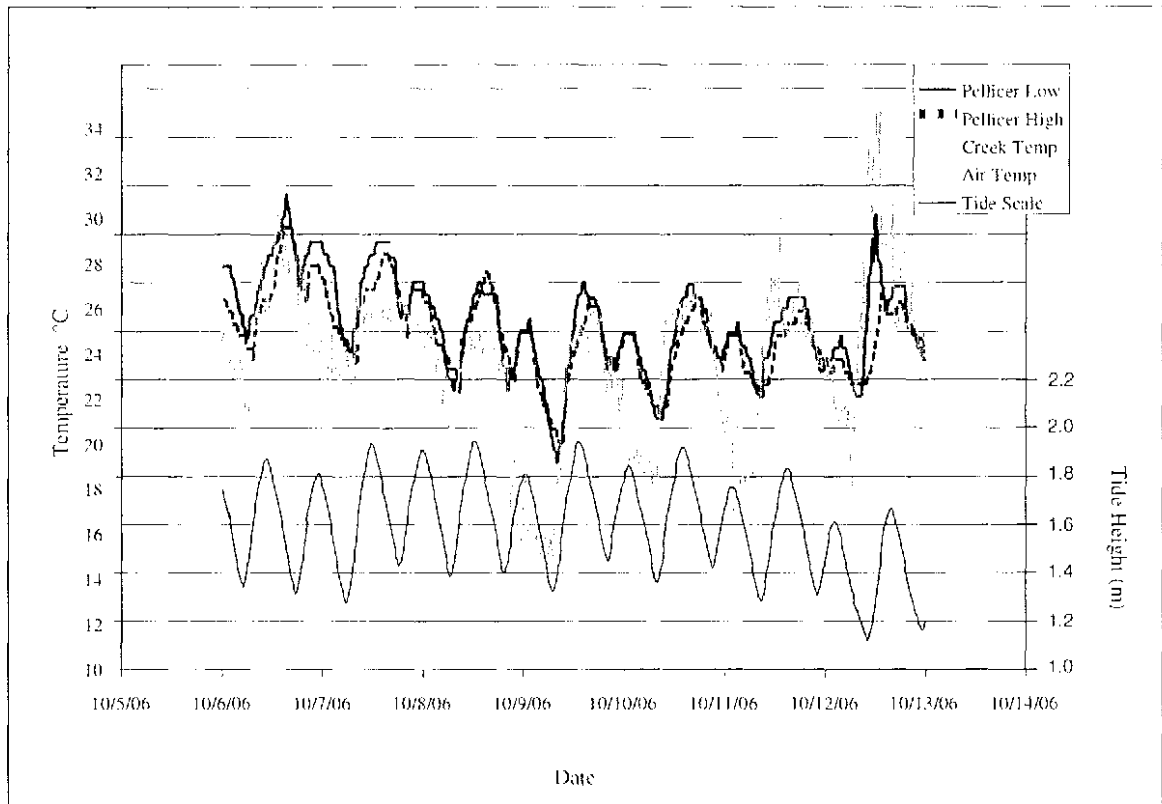
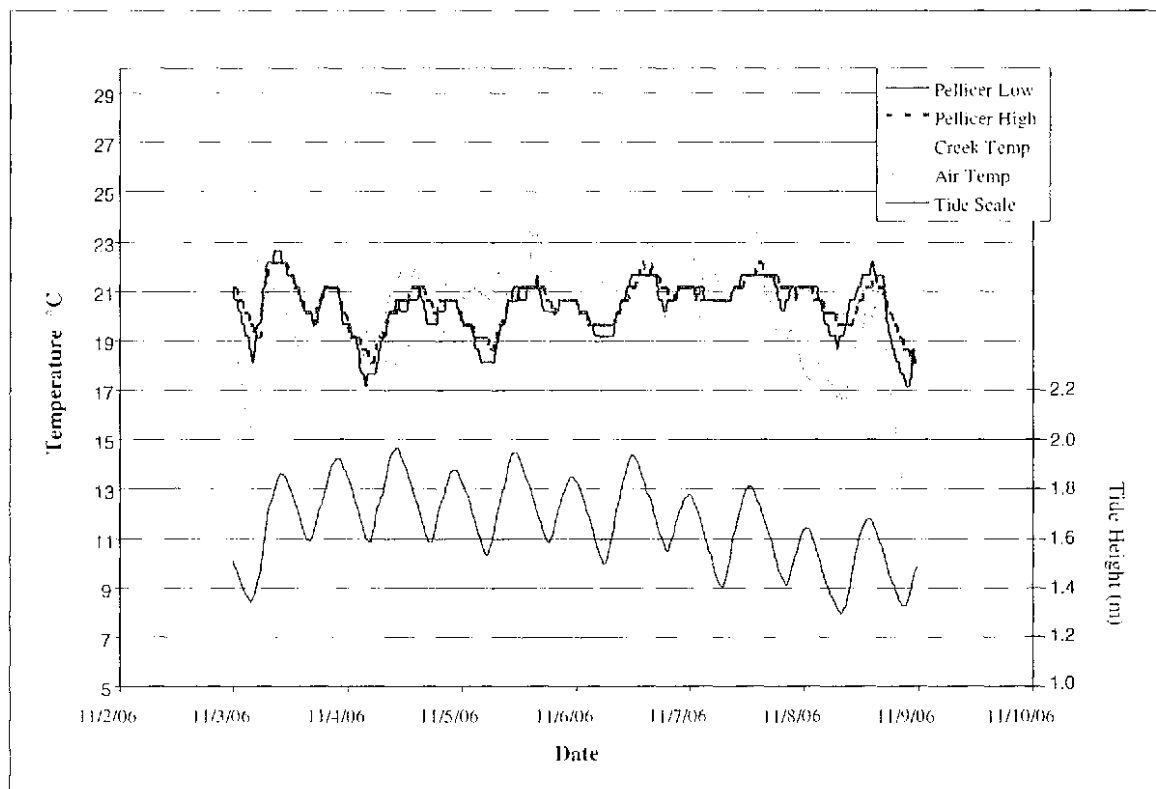


Figure 6 – Second test period for Pellicer Creek from November 3-9, 2007. Tide, air and water temperature data from permanent data sonde located at the mouth of Pellicer Creek. Pellicer low and high elevation sites located at 29.6797363N, -81.2245700W.



## *Discussion*

### *Size distribution between elevations*

Differential length distribution between elevations of the marsh for all locations studied shows size selectivity in marsh use. Smaller fish use higher elevation areas, further away from the marsh edge/rivulets, which are less populated. All sizes of *F. heteroclitus* are less abundant in the high marsh, further away from a creek in more severe habitat, showing their dependence on the daily tidal inundation. Larger juveniles were found closer to the main creek during low tide, most likely due to a greater risk of stranding on high marsh, versus smaller fish which are able to sustain themselves in a few centimeters of water (Kneib, 1984; pers. obs.). By utilizing marsh that is inaccessible by larger fish, i.e. high marsh habitats, small juveniles and larvae may be better able to maximize foraging and avoid predation (Kneib and Wagner, 1994). Smaller fish may experience more severe conditions and costs associated with physiologically adapting to those conditions. However, the benefit of accessing the marsh surface and increased foraging time is necessary for full growth (Weisberg and Lotrich, 1982). Higher elevations may provide protection from conspecific predation however, predation by xanthid crabs, other invertebrates, and birds is still prevalent (Kneib, 1984; Able et al., 2007). Larval mortality is high and entire cohorts may be lost due to abiotic and biotic events such as rainstorms, high temperatures, competition and predation (Kneib, 1984). Due to the high mortality rate of larvae and the methodology of this study, results could have been biased due to cohort and abiotic conditions following hatching. However, this study shows that Northeast Florida marshes display similar trends to previous studies of



captured as a result. Since population differences have been shown in this study, further experimentation with more locations within a single population are needed to compare factors that may affect distribution of *F. heteroclitus* in the Northeast Florida salt marshes.

#### *Abiotic conditions*

Many abiotic and biotic factors beyond the scope of this exploratory study may have determined the distribution of larval *F. heteroclitus*. Although sites were chosen specifically for physical similarity, small scale differences were present between sites. Temperature differences at the Pellicer Creek site were not great enough to account for the greater abundance of fish on the lower marsh than the higher marsh. However, a complete temperature comparison over the mating season from all locations could show different results that were not present in the shorter sub-sampling time frame.

Differences seen in populations may have been due to the abiotic and biotic differences in locations and phenotypic plasticity exhibited by the fish rather than true differences between populations. Tide height differences between locations may have been a factor in fish abundance, with the Pellicer Creek high site having the lowest abundance of fish and the smallest maximum tidal inundation. Further studies using small data recording devices able to detect various abiotic characteristics in local area marshes could help the broader understanding of large scale marsh use patterns. Elucidating some of the factors that determine the distribution of organisms on the marsh from nekton to the interstitial invertebrates could be helpful in determining the overall value of a marsh.

## Chapter 3 Comparison between *Fundulus heteroclitus* and *F. grandis* larval thermal maxima

### *Introduction*

Organisms are adapted to inhabit only a small range of biological parameters that exist. Within those parameters, abiotic and/or biotic interactions limit how much tolerable range the species occupies. Patterns of large scale distribution may be determined by examining what factors confine a species to its realized niche. Examining specific tolerances to abiotic and biotic factors may help determine which of those factors influence a species' realized niche and limits its distribution.

The family *Fundulidae* has shown a propensity for many of its species to share overlapping distributions. Because it is a commonly studied laboratory organism and much is already known about its physiology, ecology and genetics, it is an ideal study system (reviewed by Atz, 1986 and Powers et al., 1986). A bulk of these studies have been performed on the Common killifish, *Fundulus heteroclitus*, used for its ubiquity in salt marsh habitat, hardiness, and high fecundity. *F. heteroclitus* has a wide distribution, overlapping many different species of Fundulids, and in some cases creating different hybrid zones (Able and Felley 1986; Wiley 1986). Previous literature that focused on hybrid zones has shown that Fundulid distribution is bound by many factors that affect both species. Specifically, *F. heteroclitus* has two sub-species *F. heteroclitus macrolepidotus* and *F. heteroclitus heteroclitus*, which are separated by geographical locations. The two sub-species from north and south of the Chesapeake and Delaware

bays respectively, have significant differences in egg characteristics and spawning behavior (Morin and Able 1983; Able 1984). Other species of Fundulids, *F. majalis* and *F. similis* have possibly formed hybrid zones across an ecotone separated by habitat type as the marsh changes from northern *Juncus* – *Spartina* marsh to southern mangrove marsh (Duggins et al. 1995).

*Fundulus heteroclitus* and *F. grandis* are resident salt marshes species that inhabit tidal creeks and flats. *F. heteroclitus* are mainly distributed along the Atlantic coast of the U.S. from Maine to Northeast Florida (Kneib 1986). Gulf killifish, *Fundulus grandis*, range throughout the Gulf of Mexico and east around Florida to the Atlantic coast (Lipcius and Subrahmanyam, 1985). The range of *F. heteroclitus* and *F. grandis* is thought to overlap just south of Jacksonville, Florida (Duggins et al. 1995, Gonzalez, pers. comm.). The tendency of Fundulid fish to live within close vicinity of each other, their likely resource overlap, and observations in the field lead to the hypothesis that *F. heteroclitus* and *F. grandis* should have some interaction, either abiotic or biotic, that limits their distributions from continuing south and moving north respectively. Although species are often separated by a combination of biotic and abiotic conditions, due to the ease of laboratory manipulation this exploratory study focuses only on the abiotic conditions.

#### *Life history*

Some juveniles display differences in physiological tolerance to abiotic conditions than conspecific adults. In *Menidia menidia*, newly hatched larvae and two week old fish were more tolerant to heat shock and showed no significant difference in predator avoidance after heat shock than four week old larvae (Deacutis, 1978). Young *Gambusia*

*affinis* are found at and prefer higher temperatures than adults; this is thought to reduce intraspecific predation and competition (Bacon et al., 1967). Juvenile *Poeciliopsis occidentalis* and *P. monacha* have a higher tolerance to heat stress than adults of the same species (Bulger and Schultz 1982). These examples of heat tolerance in juvenile fish show the possible age groups differences in tolerance to abiotic conditions.

*Fundulus heteroclitus* and *F. grandis* are resident salt marsh species, and their life history exposes them to wide ranges of abiotic conditions that occur in the harsh tidal dependent habitat (Lipcius and Subrahmanyam 1985; Kneib 1986; Weisberg 1986). Adult killifish predominantly reside in tidal creeks and access the marsh surface during high tide to feed. However, larvae utilize natural depressions on the marsh surface between the emergent *Spartina alterniflora*. These shallow depressions are susceptible to more extreme abiotic conditions because they are only flushed during high tide; some only flushed during spring tides where tidal amplitude is greatest. Infrequent inundations can lead to a variety of abiotic extremes such as higher water temperature and lower dissolved oxygen, especially during summer months; higher salinity due to evaporation, or low to no salinity due to precipitation. Therefore, it has been hypothesized to utilize this extreme habitat juveniles would exhibit a greater tolerance to extreme abiotic conditions.

#### *Abiotic factors considered*

Examining a single abiotic factor is a necessary first step in elucidating the role, if any, of that factor in the distribution of an organism (Sylvester, 1975). Both *F. heteroclitus* and *F. grandis* adults exhibit high tolerances to a large range of the major abiotic characteristics: salinity, dissolved oxygen, and temperature. Both fish are

categorized as euryhaline which means that they can tolerate a wide variety of fluctuating salinities as opposed to stenohaline fish that only survive in a narrow range of salinities. Adult *Fundulus* not only tolerate a large range of salinities, but also commonly inhabit waters throughout their range. *Fundulus grandis* have been collected from waters of 0.05 – 76.0 ppt salinity (Perschbacher et al. 1990, Simpson and Gunter 1956). Adult *F. heteroclitus* have a salinity tolerance from 0.0-120.3 ppt (Griffith 1974), and nine day old larvae have a range of 0.83 – 40.14 ppt with no mortality with a maximum range of 0.83 – 102.05 ppt with 50% mortality (Joseph and Saksena, 1966). Dimichele and Taylor (1980) found that salinity within a range of 0-30 ppt does not show a significant effect on hatching rate of *F. heteroclitus*. Natural changes in salinity due to evaporation or precipitation will most likely fall within the tolerable range and not greatly affect the juvenile fish. Therefore, salinity will not be manipulated in this study, although field salinity will be recorded and examined along with other abiotic characteristics.

Dissolved oxygen (DO) can play a large role in fish distribution and survival. Oxygen tolerances are often measured by the end point of aquatic surface respiration (ASR) where a fish moves to the surface to reduce effects of low DO. Adult *Fundulus grandis* summer oxygen tolerance is 0.48 ( $\pm 0.10$ ) mg/l with the highest low oxygen tolerance of 0.81 ( $\pm 0.11$ ) mg/l in the fall (Love and Rees, 2002). *F. heteroclitus* young of the year (under 1 year old) exhibited ASR at a DO of 1.09 ( $\pm 0.02$ ) ppm and had 50% mortality at 0.23 ( $\pm 0.02$ ) ppm (Smith and Able, 1994). Again, because previous literature has already shown a large tolerance to nearly anoxic water, oxygen is not likely to affect the distribution of both species.

Temperature, as a single factor, could cause thermal partitioning between species within a given habitat (Sylvester 1975). Adult *F. heteroclitus*' upper lethal limit of temperature is 36.3°C (Garside and Chin-Yuen-Kec 1972; Table 5). Acclimated at 34.0°C (Fangue et. al 2006) and 36.0°C (Bulger, 1984), the critical thermal maximum for adults was 42.5°C ( $\pm 0.96$ ) and 44.11°C ( $\pm 0.135$ ) respectively. Fundulids exhibit a high tolerance to many abiotic factors however, laboratory experiments will be limited to temperature, which has no known reports of non-adult tolerance levels and is considered by some to be the abiotic master factor (Fry 1947). Additionally, Middaugh et. al (1978) demonstrated a significant effect of thermal stress on the developmental rate in four larval stages of *F. heteroclitus*. Differences in temperature-specific development rates were seen between northern and southern populations of *F. heteroclitus*, most likely due to genetic controls (DiMichele and Westerman, 1997). Since fish are ectotherms, most of their biochemical, physiological and life history activities depend on, and are regulated by, the surrounding water temperature (Beitinger, et al. 2000). Due to this control of the physiological activities, temperature can act as a lethal factor that is easily manipulated and quantified in a laboratory setting.

Shifts in habitat use throughout the day may play a key role in habitat partitioning and in behavioral responses to abiotic conditions. Previous studies indicate some species utilize diel cycles to partition resources within the same habitat. Physiological tolerances can have environmental as well as genetic components and could be affected by circadian rhythm. Both male and female *Gambusia affinis affinis* show diel variation with a thermal peak between 1000 and 1300hr, coinciding with an increased activity level (Johnson 1976). Circadian rhythm was found to affect the activity levels of groups of *F.*

*heteroclitus* (Kavaliers, 1980). Bulger (1984) shows significant differences in temperature tolerance of *F. heteroclitus* throughout the day, with the highest thermal maximum for summer acclimated fish at mid day, in the presence or absence of light during experimentation. If this pattern, as seen in adults, has a genetic basis, then larval fish should also show mid day thermal peaks regardless of their thermal history. If this pattern is not seen in larvae, then it is probably due to a history of daily fluctuating temperatures or another external cue, such as photoperiod, that affects the physiological response in the adults. This study will examine diel patterns in thermal tolerance for larval fishes of both *F. heteroclitus* and *F. grandis*.

#### *Objectives*

The objective of this study is to determine if thermal partitioning is a plausible explanation for the distribution pattern between *F. heteroclitus* and *F. grandis* by quantifying larval thermal maximums and determining if patterns exist between species, populations, and/or time of day. Also, to determine how the larval thermal tolerance compares to temperatures experienced in nature.

Table 5 – Previous literature of adult *F. heteroclitus* thermal tolerance data. CTM – critical thermal methodology, LOE – loss of equilibrium, ULT – upper lethal temperature.

Acclimation (°C)	Method	Endpoint	Time or Rate	Maximum Temp. °C	Location	Reference
30	ULT	Death, opercular spasms	5° per hour up to 35 then 2° per hour down	41.7(0.55)		Burnside (1977)
25	ULT	Death	1°C per	36.31 (0.19)	Nova Scotia	Garside and Chin-Yuen-Kee (1972)
30	CTM	LOE	0.3°C per min	43.08 °C (0.239)	Williamsburg, Virginia	Bulger (1984)
34	CTM	LOE	0.3°C per min	43.6 °C (0.20)	Williamsburg, Virginia	Bulger and Tremaine (1985)
36	CTM	LOE	0.3°C per min	44.11 °C (0.135)	Williamsburg, Virginia	Bulger (1984)
32.1 (0.44)	CTM	LOE	0.28-0.33°C per min	42.4°C (0.84)	Georgia and Florida	Fangue et. al (2006)
34.0 (0.32)	CTM	LOE	0.28-0.33°C per min	42.5°C (0.96)	Georgia and Florida	Fangue et. al (2006)



## Methods

Adult fish were caught by trap or seine from Florida *F. heteroclitus* populations in St. Mary's River (30.7189600, -81.4733000), Nassau River (30.5209850, -81.4986218); and *F. grandis* populations in Cedar Key (29.1420000, -83.0356700) and Indian River Lagoon (28.6767971, -80.7717949; Figure 7). Fish populations were held in four 95L tanks in a flow through system for at least four weeks with the St. Marys River population held for four months. Fish were kept at a photoperiod of 14 lt:10 dk in room temperature water roughly 25-27°C with a salinity of approximately 25 ppt. Fish were fed once daily a diet of Tetramin™ pellets intermittently supplemented with grass shrimp, *Palaemonetes pugio*, and various chopped fish. Fish were spawned in the lab generating offspring of each species within their respective populations. Gametes were harvested from mature female fish by applying pressure from the abdomen towards the anus expelling developed eggs and from male fish by harvesting and mashing testes. Gametes were collected separately from a minimum of 2 individuals and up to 7 individuals of each sex and were then combined into a single mass spawning within each population. Fertilized eggs were kept submerged in brackish (15 ppt) in approximately 29°C water in 10 cm glass bowls for development. Acclimation temperature was chosen due to technical difficulties with the laboratory air conditioner where roughly half the eggs experienced a water temperature fluctuation of  $\pm 3^{\circ}\text{C}$  for four days and then drop 5°C before all eggs could be moved into an incubator. Water was changed every two days and unfertilized or defective (cloudy) eggs were removed. At 12 days post spawning, eggs were drained of water for 1 hour then reimmersed to cue hatching (DiMichele and Taylor 1980). Eggs that failed to hatch were cued again once a day and

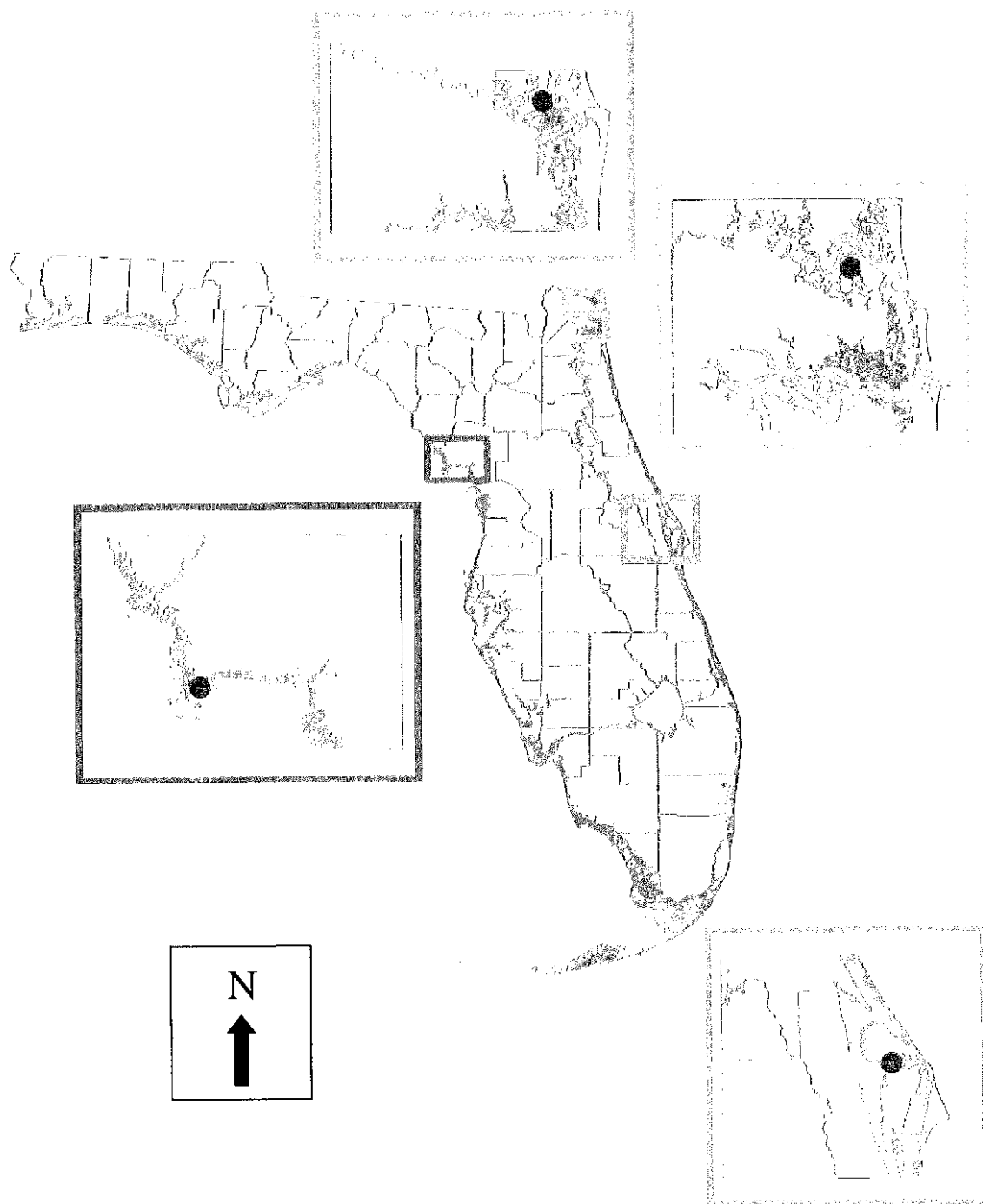
then discarded if they failed to hatch by 20 days post-spawning. Once hatched, larvae were housed in 10 cm depth x 5 cm height pyrex containers in an incubator under a photoperiod of 14 lt:10 dk, fed Artemia, and had at least 50% water changed daily. Feeding and water changes were performed at various times throughout the day to avoid thermal routine. Larval fish were acclimated to approximately 29°C and 20 ppt salinity over a period of 9 days post hatching. Larval size classification is determined by absorption of the yolk sack, roughly 3 days post spawning, and up to a standard length (SL) of approximately 10-11mm or 13.5mm (Talbot and Able, 1984; Kneih, 1986).

Larval fish thermal tolerance was estimated using Critical Thermal Methodology (CTM) to examine differences among populations and between species against the known values for adults. CTM exposes fish to a constant rate increase or decrease of water temperature, slow enough to allow body temperature to equalize but fast enough to avoid acclimation, up to either a lethal or non-lethal endpoint (Becker and Genoway, 1979). Nine day old larvae were chosen haphazardly from each population and placed into aerated containers suspended in a circulating water bath. The water temperature was increased at a mean rate of  $0.27 (\pm 0.04) ^\circ\text{C}/\text{min}$  until fish exhibited Loss of Equilibrium (LOE). LOE is defined as an ecological death point where dorsal-ventral orientation cannot be maintained preventing organisms from escaping current conditions (Becker and Genoway 1979; Bennett and Beitinger 1997; Beitinger, et al. 2000). Final water temperature and standard length was recorded for all fish after which the fish were returned to ambient starting conditions to recover. Multiple trials were run in the morning; 7am-9am, afternoon; 11am-1pm, and evening: 4pm-6pm for each of the 4 populations of fish, with individual fish participating in only one trial. Critical thermal

maximums were calculated as the arithmetic mean for each group and analyzed by ANCOVA ( $\alpha=0.05$ ) to determine differences between populations, species and time of day. Within population differences across time were analyzed by ANOVA to determine whether CTMax values change throughout the day as shown in adults (Bulger 1984).

Field temperature was determined from Sept. 30 – Nov. 21, 2006 for the Pellicer Creek location. Temperature was measured continuously every 15 min from a 10 cm diameter x 5 cm height pyrex crystallization dish dug into the marsh and held flush to the surface by 3 metal stakes, to simulate a natural marsh depression. The dish was located near the spring high tide mark but close enough to a marsh creek or small rivulet to be completely inundated during spring high tide, in an area with *Fundulus* larvae on the surface. Temperature was recorded using an ibutton temperature sensor and digital data logger (model number DS1992L-F50). The ibutton was suspended from a mesh covering the dish to reduce the effects of sediment contaminating the temperature readings. Continuous tide, water and air temperature data were collected from an established data sonde maintained by Guana Tolomato Matanzas National Estuarine Research Reserve (GTMNERR) located at the mouth of Pellicer Creek to cross-reference data and examine trends in temperature data recorded from traps. Due to a lack of permanent data sondes and technical difficulties no other sampling locations were used to determine field temperature data.

Figure 7 – Location of adult populations sampled for larval thermal tolerance trials. *F. heteroclitus* populations: St. Mary's River (30.7189600, -81.4733000), Nassau River (30.5209850, -81.4986218); *F. grandis* populations: Cedar Key (29.1420000, -83.0356700), Indian River Lagoon (28.6767971, -80.7717949).



## Results

### *CT*Maximum temperature tolerance

Mean LOE temperatures ranged from 43.04 – 43.65 °C (Figure 8). Significant differences in LOE temperatures among populations were observed after removing variance associated with significant accessory factors such as hatch date and heating rate (Table 6). LOE temperatures from both populations of *F. heteroclitus* were not significantly different from each other (Table 7). Between species, *F. heteroclitus* from Nassau had significantly higher mean LOE temperatures than either *F. grandis* population (Table 7) but, *F. heteroclitus* from St. Mary's River had significantly higher LOE than *F. grandis* from Indian River but were not different from Cedar Key. Conversely, both populations of *F. grandis* were significantly different from each other, with Cedar Key fishes displaying a significantly higher mean LOE temperature than Indian River fishes. Mean standard lengths were not normally distributed ( $p < 0.01$ ), but when factored as a covariate, length was not found to be significantly different among populations (Table 8). Significance of hatch date could be due to the mechanical failure in the laboratory causing incubation temperature varied drastically. The acclimation period of 9 days post hatching was thought to be sufficient to eliminate thermal differences but the incubation temperature was most likely still a significant factor. Since there were differences between populations, data could not be pooled to determine if thermal tolerance differences exist between species. However, the general pattern suggest that *F. heteroclitus* has a slightly higher thermal tolerance than *F. grandis* (Figure 8)

### *Time of day*

Time of day did not significantly affect LOE (Table 6) but since the ANCOVA model removes variance associated with all factors, it is very conservative and may not be able to detect small differences. However, when examined separately, no significant mid-day thermal tolerance peaks were observed in any population. Nassau was the only population to have significant differences due to time of day, showing a lower LOE during evening, than either morning or afternoon (Figure 9, Table 9 and 10). Mean LOE temperatures appeared to differ less between populations of the same species than between conspecific populations at all times except for the morning. Again, since there were significant differences among populations the results could not be pooled to determine if LOE temperatures differ between species throughout the day. Further experimentation at closer increments of time was performed to determine if any small scale patterns of LOE temperatures occur throughout the day. Only Nassau fish were used to represent *F. heteroclitus* due to the low sample size from the St. Mary population. Neither *F. grandis* population was manually fertilized, but instead already fertilized eggs were obtained from the aquaria 3 days after manual spawning had been unsuccessful. Already fertilized eggs should have little thermal difference from manually fertilized eggs since they were only housed in the tanks for about 1 day and then held under the same incubation conditions as previously stated. *F. heteroclitus* seem to show more variability than *F. grandis* with a 0.6°C difference between the highest and lowest mean LOE temperature throughout the day, as opposed to 0.29 and 0.26°C from Indian River and Cedar Key, respectively (Figure 10).

### *Field temperature*

Field temperature data during the reproductive season for the Pellicer Creek site was found to be below the thermal tolerance of juvenile fish, with the exception of 2 hours, between 14:30-16:00, on July 5, 2006 where the dish temperature spiked to 47.1°C (Figure 11). The surrounding water temperature from the data sonde was well below the dish temperature at 33.0°C with air temperature even cooler at 31.3°C. Therefore, it is likely that the temperature spike is a result of sediment contamination insulating the ibutton and surface radiative warming by the sun.

Table 6 – ANCOVA for *F. heteroclitus* from St. Mary, Nassau, Cedar Key and Indian River populations with hatch date and heating rate as covariates. Significant factors shown in bold.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	834.591	1	834.591	5457.260	0.000
	Error	26.235	171.544	0.153		
Length	Hypothesis	0.114	1	0.114	0.746	0.389
	Error	25.950	170	0.153		
hatch_date	Hypothesis	2.623	1	2.623	17.182	<b>0.000</b>
	Error	25.950	170	0.153		
heat_rate	Hypothesis	1.154	1	1.154	7.560	<b>0.007</b>
	Error	25.950	170	0.153		
Population	Hypothesis	1.615	3	0.538	2.904	<b>0.044</b>
	Error	8.683	46.851	0.185		
Time of Day	Hypothesis	1.233	5	0.247	1.170	0.358
	Error	4.280	20.301	0.211		
Population * Time of day	Hypothesis	3.204	14	0.229	1.499	0.116
	Error	25.950	170	0.153		



Figure 8 – Population effects of Loss of Equilibrium (LOE) on fish acclimated to 29°C. *F. heteroclitus* from St. Marys (SM) 43.42 ( $\pm 0.58$ ) n=29 and Nassau (NA) 43.65 ( $\pm 0.55$ ) n=71. *F. grandis* from Cedar Key (CK) 43.31 ( $\pm 0.27$ ) n=30 and Indian River (IR) 43.04 ( $\pm 0.36$ ) n=75. Significant groups indicated by letter(s).

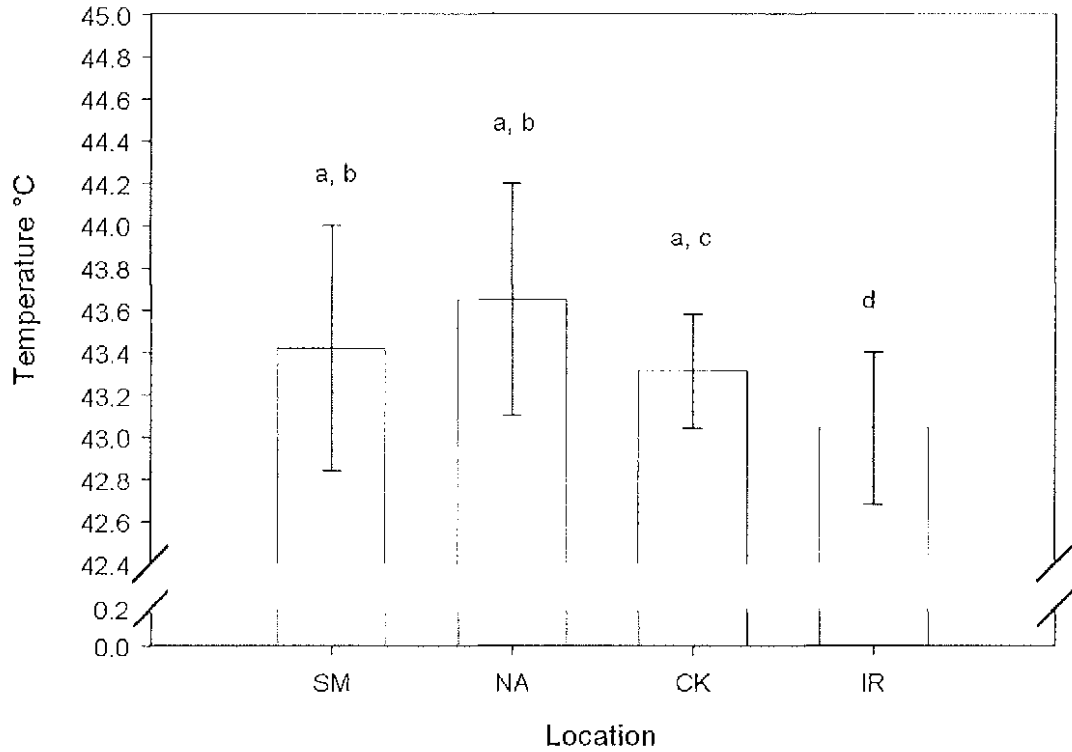


Table 7 – Tukeys HSD pairwise comparisons of differences between populations of *Fundulus heteroclitus*, St. Marys and Nassau; and *F. grandis* Cedar Key and Indian River.

	St. Marys	Nassau	Cedar Key	Indian River
St. Marys	1.000			
Nassau	0.105	1.000		
Cedar Key	0.758	<b>0.003</b>	1.000	
Indian River	<b>0.001</b>	<b>0.000</b>	<b>0.042</b>	1.000

Table 8 – Standard Length (mm) of *Fundulus* hatched in the laboratory. One sample Kolmogorov-Smirnov test  $p < 0.011$ . *Fundulus heteroclitus*, St. Marys and Nassau; and *F. grandis* Cedar Key and Indian River.

Population	SL range (mm)	Mean	SD
Nassau	5.7 – 8.2	6.62	0.42
St. Marys	6.3 – 8.3	7.36	0.51
Indian River	7.0 – 8.4	7.81	0.30
Cedar Key	6.6 – 8.9	7.64	0.36

Figure 9 – Comparison of time of day thermal tolerances between populations. *Fundulus heteroclitus*, St. Marys n=10, and Nassau n=24; and *F. grandis*, Cedar Key n= 11, and Indian River n=25 for each hour with standard error bars shown.

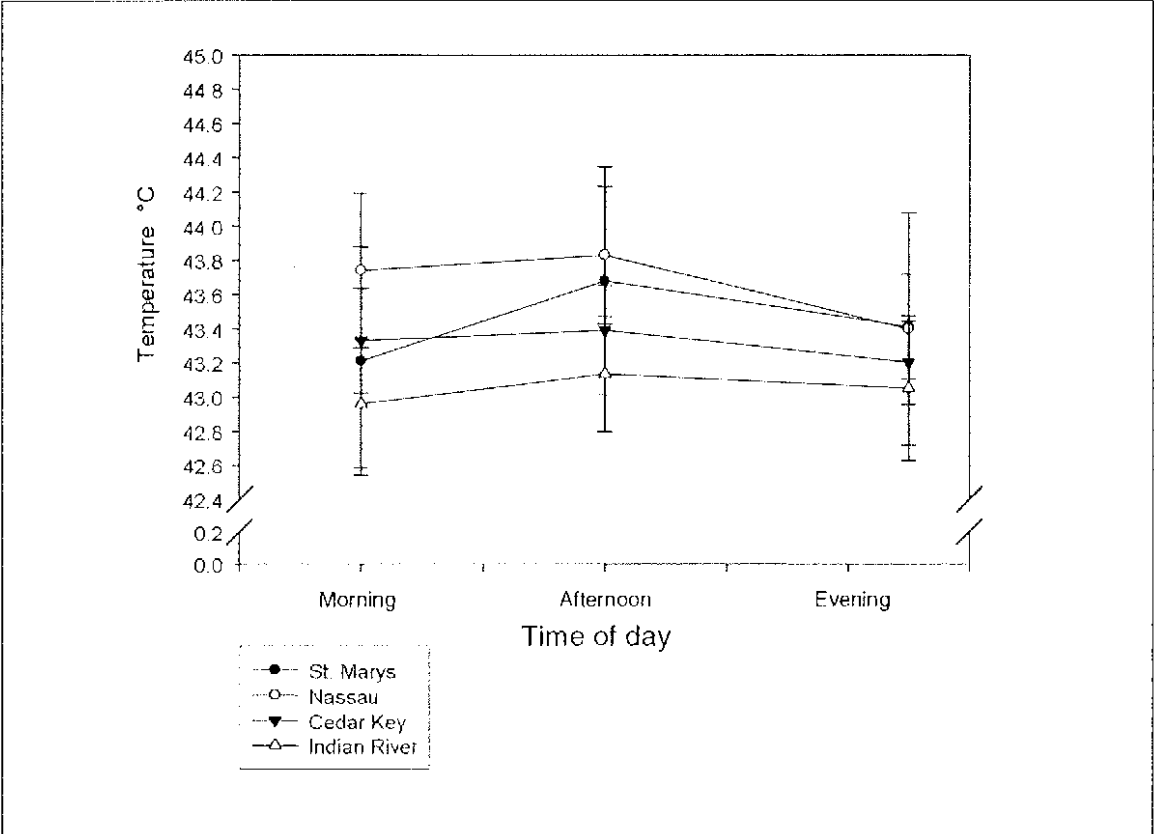


Table 9 – ANOVA table for differences of LOE through out the day within populations. Significance shown in bold

Source		Sum of Squares	df	Mean Square	F	Sig.
St. Marys	Hypothesis	1.040	2	0.520	1.602	0.221
	Error	8.434	26	0.324		
Nassau	Hypothesis	2.378	2	1.189	4.315	<b>0.017</b>
	Error	18.738	68	0.276		
Cedar Key	Hypothesis	0.385	2	0.192	0.770	0.465
	Error	30.504	122	0.250		
Indian River	Hypothesis	0.386	2	0.193	1.539	0.222
	Error	9.039	72	0.126		

Table 10 – Nassau population LOE significance due to time of day. Tukey HSD post hoc test.

	Morning	Afternoon	Evening
Morning	1.000		
Afternoon	0.834	1.000	
Evening	0.060	0.0220	1.000

Figure 10 – Comparison of thermal tolerances throughout the day between populations. *F. heteroclitus* from Nassau n=11 and *F. grandis* from Cedar Key n= 15, and Indian River n= 12 for each hour with standard error bars shown.

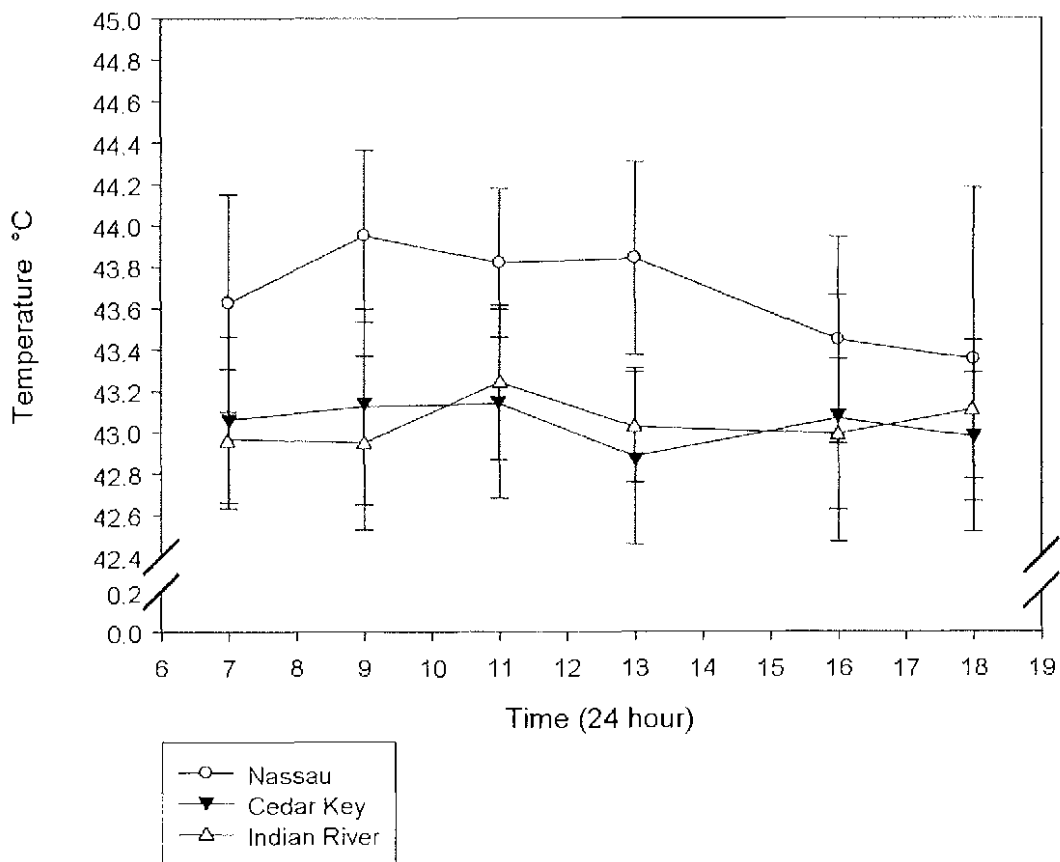
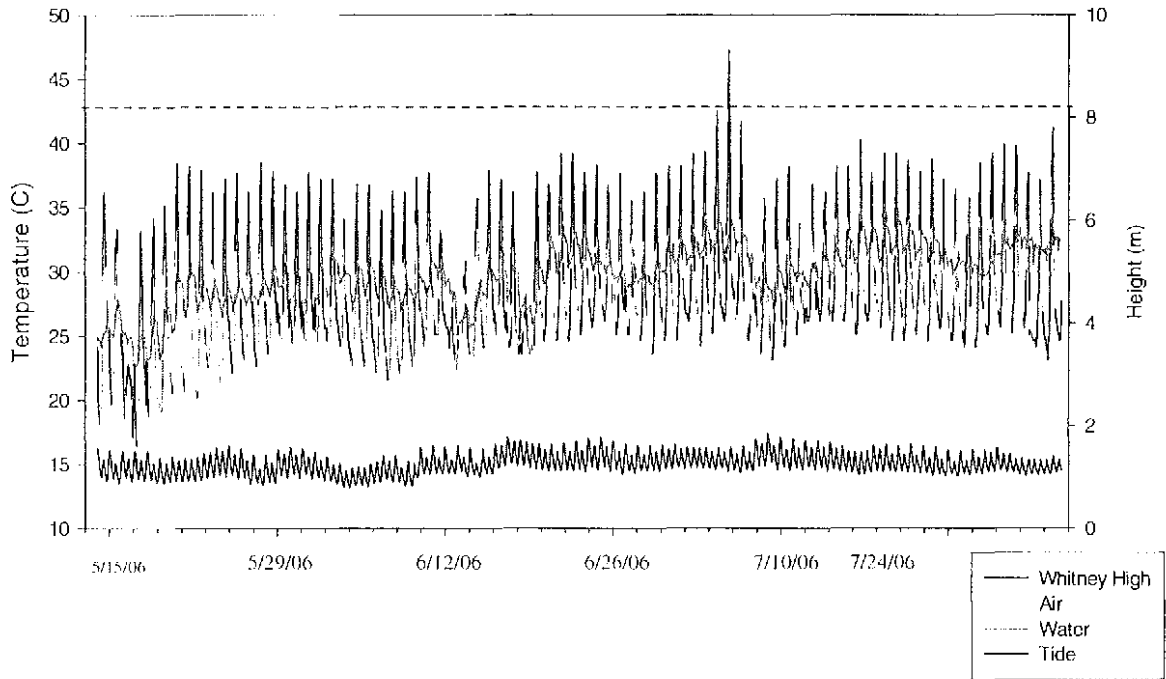


Figure 11 -- Pellicer Creek field temperature sample period. Tide, air and water temperature data from permanent sonde located in Pellicer Creek maintained by GTMNEER. Dashed line represents maximum thermal tolerance of juvenile *F. heteroclitus* (this study).





## Discussion

### *CT*Maximum differences in populations

The data presented shows juvenile fish have similarly high, and in some cases higher, thermal maximums than previously reported values for adult *F. heteroclitus* (Table 5). LOE was found by this and previous studies to be higher for juveniles than adults for *F. heteroclitus*, (Fangue et al, 2006), even when acclimation temperatures were lower. This study also confirms previous research noting *Fundulus* as one of the most heat tolerant fishes. Only ten other species exhibit a LOE over 42.0 °C (reviewed by Beitinger et. al 2000). The high thermal tolerances observed may increase predator avoidance in the hottest microhabitats at midday in the summer since fry have been observed in shallow pools at 50 °C (Bulger and Tremaine 1985)

An alternate explanation is LOE differences are due to differences in size of the fish. Size has previously been shown to affect thermal tolerance in fish (Becker and Genoway 1979 and Beitinger et al. 2000), with larger fish exhibiting LOE at lower temperatures than small fish. However, use of a moderate 0.3°C heating rate, as in the present study, should ameliorate any small scale size differences, as measured by thermal penetration times. Nassau fish had a smaller mean length than every other population including other *F. heteroclitus*, and should therefore have the highest LOE based on size following the trend seen in this study (Table 8). However, the ANCOVA model is robust enough to handle non-normal distribution, and when length differences were factored out, LOE differences based on population were still found to be significant (Table 6).

Though not significantly different, *F. grandis* seem to have an overall lower thermal tolerance than *F. heteroclitus*. This is counterintuitive since *F. grandis*

experience higher temperatures than *F. heteroclitus* in nature and previous comparisons between Northern *F. h. macrolepidotus* and *F. h. heteroclitus* show greater temperature tolerance in the more southern sub-species (Fangue et al. 2006). Southern embryos develop slower and have a higher upper lethal limit than northern populations (DiMichele and Westerman, 1997). However, lower thermal tolerances may be explained by the variance associated with the thermal environments. A study of Orangethroat Darters, found that fish in more thermally variable streams showed a higher thermal tolerance than fish with less thermal variance (Strange et. al 2002). The study also found that even though gene flow could occur between environments, differences in CTMaxima and growth still exist, perhaps due to localization. Other studies with *Cyprinodontids* found in thermal pools also show this relationship (Brown and Feldmeth, 1971; Feldmeth et al., 1974). It is difficult to assess the daily thermal environment of juvenile *Fundulus* because of the harsh conditions presented by the daily tidal cycle. Little research has been done on modeling temperatures in shallow depressions on the marsh surface. (Ch. 2 this study)

The significant differences in thermal maxima among populations are intriguing since many previous studies have shown the opposite effect (Otto, 1973; Fields et al., 1987; Strange et al., 2002; Fangue et al. 2006). In previous studies, no differences in heat shock proteins were found between populations of *F. heteroclitus* from Georgia and North Florida (Fernandina Beach; Fangue et al. 2006), and specifically in this study, St. Mary and Nassau populations, a much shorter distance apart, exhibited similar thermal maximums. However, it does not explain why St. Mary fish are not significantly different than Cedar Key fish which inhabit different bodies of water and have no chance for gene flow and therefore little possibility for physical or genetic similarity. One

possible explanation could be the fact that the St. Mary fish were held in the laboratory for twice as long as any other population and the fish hatched were not a true genetic sample of the population.

Critical thermal maximum temperatures show that both species can tolerate roughly the same extreme high temperatures and, based on temperature alone, should be able to live in the same habitat. The question, therefore, still remains as to what is limiting the range of these species. It is possible that minimum temperatures or the complete thermal scope, instead of the maximums investigated here, may play a role in thermal partitioning. Acclimation temperature has the greatest affect on CTM trials with increases in acclimation temperature most often leading to increases in thermal maximums (Beitinger and Bennett, 2000). Because acclimation temperature plays such a vital role in thermal tolerance, populations or species differences may arise when tested at various acclimation temperatures including the highest possible acclimation temperature without death, found for *F. heteroclitus* to be  $< 40^{\circ}\text{C}$  (Bulger and Tremaine, 1985). Although thermal partitioning has not been completely ruled out, it is unlikely that this is the sole reason for separation between these two species. Temperature is likely an important factor in differentiating heat shock proteins and possible latitudinal genetic differences between populations and sub-species of *F. heteroclitus* (Powers et al. 1986). However, it does not appear to be the distinguishing difference between *F. heteroclitus* and *F. grandis*. Temperature controls virtually all physiological aspects and may have an additive affect influencing the outcome of a biotic interaction resulting in habitat partitioning and limiting distribution.

Because hatch date was found to be significant in this study, further tests should be performed to determine if the effect of early hatch date on temperature tolerance is genetic or related to a water temperature spike during development. The thermal environment and thermal history during development could be major components of *Fundulus* overall thermal tolerance. When re-exposed to high temperatures, some species have an increased tolerance as seen in repeated CTMax trials (Hutchinson, 1961 and Beitinger et al., 2000). Therefore, eggs that experience high temperatures during development may have increased thermal tolerance when re-exposed as juveniles. If thermal environment does not have a significant effect on the relationship between hatch date and thermal tolerances, then a genetic component is probable. Since more individuals are produced than can survive, differential survival and reproduction allows some of those individuals to reach maturity. Early hatching fish may experience higher temperatures if spring tides do not fully inundate the marsh and ameliorate the effects of mid-day solar radiation on the shallow depressions. Also, *F. heteroclitus* are piscivorous and have been known to cannibalize their own cohorts. Larger, faster growing fish may have an advantage in not becoming prey to a conspecific (Able et al. 2007). If there is a high reproductive advantage of early hatching, where individuals are able to obtain better or more resources to grow larger faster, and a higher thermal tolerance is needed to survive in waters before the spring tide, then selection should act strongest on individuals that possess both phenotypes together.

#### *Time of day and environmental temperature*

The study by Bulger (1984) although elegant, does have one major criticism of using a single population. Given the fact that the data presented in the present study

showed significant differences in LOE among populations, Bulger's results may be skewed due to population effects and therefore not hold true for all populations of *F. heteroclitus*. The thermal peak, previously shown in adults, is either non-existent for the populations in this study, or is not yet developed in 9-day-old larval fish. Environmental factors, such as daily fluctuating temperatures could cue the fish to establish a daily tolerance rhythm. Over the span of a day, temperature follows a diel pattern with the movement of the sun, with maximum temperatures most often in the afternoon and cooler temperatures during early morning and night. Temperature tolerance patterns may be affected by exposure to a daily maximum temperature. Pupfish, *Cyprinodon amargosae* were found to significantly increase their thermal scope by 2°C when acclimated to cycling temperatures ranging from 15-35°C (Feldmeth et al., 1974 and Brown and Feldmeth 1971). Also, *Gambusia affinis affinis* displayed significantly higher CT<sub>Maxima</sub> when acclimated to cyclic temperature regime (Otto, 1974). To further test this, experiments with constant and cycling temperature acclimation and/or development periods could be performed to see if the physiological response is developed in juvenile fish. Also, day length manipulations could be made to examine circadian rhythm effects on juvenile fish and to determine if they are a cue for a daily pattern.

A possible reason for the lower variability in LOE at different times of the day in *F. grandis* compared to *F. heteroclitus* is that *F. grandis* may experience less variable field temperatures than *F. heteroclitus*. Less variable thermal environments could lead to the fish being less eurythermic and show a slightly more restricted stenothermic response. Also, *F. grandis* may have a smaller acclimation temperature range than *F. heteroclitus* and a narrow thermal scope, the total range of thermal tolerances at all possible

acclimation temperatures. Although, diurnal patterns should be displayed at all acclimation temperatures, perhaps daily thermal tolerances are more pronounced at different acclimation temperatures. In this study, fish were acclimated near, but not at, their maximum acclimation temperature, therefore further studies examining multiple different acclimation temperatures may display a thermal tolerance difference due to time of day.

Average field temperatures larval *Fundulus* experience on the marsh surface seem to be well below their physiological thermal tolerance. This shows that larvae have no need for behavioral thermoregulation by traveling to different pools on the marsh surface and probably do not partition their habitat based on temperature alone. Although, temperature differences occur between populations and probably between species further studies modeling the temperature experienced on the marsh surface are needed to elucidate any further correlation between temperature and *Fundulus* distribution.

#### Chapter 4 General Conclusion

In the area where *F. heteroclitus* and *F. grandis* overlap, a factor or multiple factors must be keeping *F. grandis* from spreading north along the east coast of Florida and vice versa. One hypothesis is that *F. grandis* is a mangrove species and is not well adapted to *Spartina-Juncus* marshes in Northeast Florida. However, in the Gulf of Mexico, where *F. heteroclitus* are not present, *F. grandis* thrive in *Spartina-Juncus* marshes. Understanding the mechanisms that mediate competition between the two species could elucidate current species distribution. Because *F. grandis* larvae and juvenile distribution was not examined in this study, the role of elevation in habitat

partitioning and distribution between the species is unknown. In areas where both species overlap, *F. grandis* being slightly larger at all life history stages could be forced to stay at the lower marsh areas. Therefore, *F. heteroclitus* would utilize more of the high marsh, areas with possibly less resources. Biotic interactions would determine how much area each species resides in, with shifts in flooding changing the competitive advantage and niche size. Most likely a combination of factors ultimately determines the range of each species and could vary through out the area of overlap. For example, winter time temperatures may play an important role especially in more open habitats along Northeast Florida, however in thermally sheltered areas temperature may not be a factor. Because both of these species are eurytolerant to such a wide array of factors examining the boundaries between the species will be difficult. Both inhabit highly variable environments that show strong seasonal and daily fluctuations. Overall, resource partitioning could still be occurring and since it is probably not defined by temperature alone could be defined by a combination of factors. Previously temperature has been the only factor shown to affect *F. heteroclitus* and can drastically affect other abiotic characteristics. Therefore, it is likely that temperature still has a major role, in combination with other factors, in contributing to resource partitioning.

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