

## ABSTRACT

KIMBALL, MATTHEW ERIC. Using Temperature Tolerance to Predict Distribution and Overwintering Success of Lionfish (*Pterois volitans/miles* complex) on the East Coast of the United States. (Under the direction of Dr. John M. Miller)

Lionfish have been observed at multiple locations along the east coast of the United States, with the majority found between Miami, Florida and North Carolina. The occurrence of lionfish represents one of the first documented invasions of a marine fish species in the western Atlantic. Most lionfish observed along the southeast US shelf have been at depths greater than 35 m, whereas in their native range lionfish inhabit depths from shore to 50 m. One potential limiting factor in the distribution of lionfish on the southeast US continental shelf is winter water temperature. In particular, the northern and inshore distribution of lionfish is predicted to be temperature limited, with Cape Hatteras as the northernmost limit for overwintering. To examine this hypothesis, temperature tolerance studies were conducted following the critical thermal minimum protocol with death as the modified endpoint. Along with temperature at death (CTMin), observations on activity and feeding behavior were recorded. Overall mean CTMin was 9.95°C (SD = 0.86) and mean temperature at feeding cessation was 16.07°C (SD = 2.14). Rate of temperature decrease and acclimation temperature did not have a significant effect on CTMin or feeding cessation. No fish were observed eating below 13°C. When combined with February water temperatures, lionfish thermal tolerance data predicted that lionfish could overwinter on the southeast US continental shelf, with a northern limit of Cape Hatteras and successful inhabitation limited to offshore of the 13°C isotherm. Although lionfish can tolerate temperatures lower than 13°C, lower temperatures may limit overwintering by controlling feeding behavior. The continental shelf break (200 m

isobath) marks the offshore limit for lionfish on the southeast US continental shelf. The current southern limit of the invasion is not bound by temperature, as lionfish could survive but have not yet been reported on the Florida coast south of Miami. Possible reasons for the constrained southern limit may include larval and juvenile transport mechanisms along the Atlantic coast as well as the initial lionfish introduction site.

**USING TEMPERATURE TOLERANCE TO PREDICT DISTRIBUTION AND  
OVERWINTERING SUCCESS OF LIONFISH (*PTEROIS VOLITANS/MILES*  
COMPLEX) ON THE EAST COAST OF THE UNITED STATES**

by

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## **PERSONAL BIOGRAPHY**

Matthew Eric Kimball was born April 28, 1977 in Charlotte, North Carolina, to Daniel Perry and Sherry Welch Kimball. He grew up in Charlotte where he attended Providence High School, graduating in 1995. Matthew then enrolled at the University of North Carolina at Chapel Hill where he completed a Bachelor of Science degree in Biology, graduating in December 1999. After taking time off to work as a research technician at the University of North Carolina at Chapel Hill Institute of Marine Sciences, he entered the graduate program in Zoology at North Carolina State University in August 2001 to work under the direction of Dr. John M. Miller. Upon completion of the bulk of his coursework on main campus, he then relocated to the coast to conduct research at the NOAA Beaufort Lab in Beaufort, North Carolina under the direction of Dr. Jonathan A. Hare.

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## TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
LIST OF FIGURES	vii
1. INTRODUCTION	1
2. MATERIALS AND METHODS	7
3. RESULTS	15
4. DISCUSSION	20
5. REFERENCES	40

## LIST OF TABLES

	Page
Table 1. Number of lionfish, rate of temperature decline, and acclimation temperature ( $T_{ACCL}$ ) used in each experiment.	29
Table 2. Results of ANOVA examining the effect of rate of temperature decline on CTMin and temperature at feeding cessation in Experiment 2.	30
Table 3. Results of ANOVA examining the effect of acclimation temperature ( $T_{ACCL}$ ) on CTMin and temperature at feeding cessation in Experiment 3.	31



## LIST OF FIGURES

	Page
Figure 1. (A) Location of temperature loggers along North Carolina coast with bathymetry shown to 60 meters, (B) bottom temperatures recorded at each site in winter 2001-2002, (C) rate of temperature decline per day for 19 cold events recorded from sites shown in A.	32
Figure 2. Correlation between satellite-derived sea surface temperature and bottom temperature at three North Carolina bottom sites: the Naeco ( $r = 0.671$ ), Papoose ( $r = 0.796$ ), and Suloid ( $r = 0.973$ ). A 1:1 relationship is shown along with lines depicted $\pm 1^\circ\text{C}$ for reference	33
Figure 3. February bottom temperature isotherms derived from 1950-1999 climatology of the southeast United States continental shelf (from Blanton et al. in press) and satellite-derived surface temperature isotherms developed as part of this study.	34
Figure 4. Critical thermal minimum and average temperature at feeding cessation observed at initial rate of temperature decrease (Exp 1). Standard error bars are shown.	35
Figure 5. Critical thermal minimum and average temperature at feeding cessation observed at three rates of temperature decrease (Exp 2). Standard error bars are shown.	36
Figure 6. Critical thermal minimum and average temperature at feeding cessation observed at three acclimation temperatures (Exp 3). Standard error bars are shown.	37
Figure 7. Potential range for lionfish on the southeast United States continental shelf predicted from $13^\circ\text{C}$ isotherm. Offshore limit is 200 m isobath.	38
Figure 8. Location of lionfish sightings since 2000. (Lionfish location data courtesy of Paula Whitfield, NOAA Beaufort Lab).	39

## INTRODUCTION

Biological invasions are defined as the establishment of species beyond their historical range (Ruiz et al. 2000). One mechanism of biological invasion is natural range expansion (Carlton, 1989), as evidenced by the increase in number of tropical species on temperate North Carolina reef communities over the past two decades (Parker and Dixon 1998). The second mechanism of biological invasion is human-mediated introduction (Carlton, 1989; Ruiz et al. 1997), with shipping and fisheries accounting for the majority of introductions (Baltz 1991, Ruiz et al. 2000). The discovery of 367 marine taxa in the ballast water of 159 cargo ships of foreign origin in Coos Bay, Oregon illustrates the voluminous transfer capabilities of oceanic shipping (Carlton and Gellar 1993). Regardless of the mechanism, successful establishment of an invasive species depends on a suite of factors including, but not limited to, the physical environment (e.g., temperature and salinity preferences), interactions with resident species, and suitable habitat availability (Moyle 1986, Baltz 1991).

The human-mediated introduction of fishes into non-native waters has long been a global problem. The earliest recorded introduction of a fish in North American waters was in the 1600's (Crossman 1991), but the majority of freshwater, estuarine, and marine species were introduced over the past two centuries (Cohen and Carleton 1998). The increase in fish introductions was spurred by the discovery of artificial propagation techniques and the advent of more efficient transportation in the mid- and late-1800's (Moyle 1986). Most fish introductions have been of freshwater species, but recently a number of marine fish introductions have been recorded (Randall 1987, Erdmann and Vagelli 2001, Whitfield et al., 2002). The small overall number of documented marine

fish invasions has limited researchers abilities to thoroughly investigate and predict the consequences of marine fish introductions on native species and ecosystems, but based on the effects of invasions into other aquatic ecosystems, marine fish invasions have the potential to be problematic.

Lionfish (*Pterois volitans/miles* complex) are a highly visible predatory fish native to tropical coral reefs ranging from the Mediterranean Sea (Golani and Sonin 1992) to the southern Indian Ocean, and extending east to the Indo-West Pacific (Schultz 1986). Since 2001, adult and juvenile lionfish have been observed on the mid and outer shelf (20-80 m) off the coasts of North Carolina, South Carolina, Georgia, Florida, New York, and Bermuda on multiple occasions (Whitfield et al. 2002). The presence of lionfish along the eastern seaboard of the United States is thought to be one of the first successful introductions of a marine fish from the western Pacific to the Atlantic coastal waters of North America (Whitfield et al. 2002). Due to the large geographic barriers separating the native range of lionfish from the western Atlantic Ocean, natural range extension is not a plausible explanation for this introduction. As for mechanisms of human mediated introduction, all evidence supports the conclusion that the invasion of lionfish resulted from aquarium trade releases (Hare and Whitfield, in press).

*P. volitans* and *P. miles* are considered allopatric sibling species in the scorpionfish subfamily Pteroinae, genus *Pterois* (Schultz 1986), but their status as distinct species or two populations of a single species is uncertain (Kochzius et al., in press). Based on meristic and morphometric differences, the sibling species can be distinguished by dorsal and anal fin ray counts, relative pectoral fin size, and the relative size of spots on the soft vertical fins (Schultz 1986). Additionally, Kochzius et al. (in

press) separated *P. volitans* and *P. miles* on the basis of unique mitochondrial DNA sequences. However, Kochzius et al. (in press) analysis was ultimately inconclusive with regards to *P. volitans* and *P. miles* as distinct species or two populations of a single species, and they point out the need for the collection and examination of more specimens before a decisive status can be determined. Therefore, for the purposes of this study, the two species are grouped together as the *P. volitans/miles* complex and are referred to commonly as lionfish.

Juvenile and adult lionfish are generally associated with reefs from shore to approximately 50 m (Schultz 1986). Lionfish are easily distinguished by their unique striped body coloration consisting of red-brown or dark brown bars on a pale background (Schultz 1986) and large, fan-like dorsal-fin spines and pectoral rays bearing the same color pattern. As found with several other members of the Scorpaenidae family, lionfish have venomous dorsal, anal, and pelvic spines (Randall et al. 1990, Gallagher 2001). In their native range, adult lionfish tend to remain alone and stationary at one site throughout the day, while as juveniles and during reproduction, lionfish occur in small groups (Fishelson 1975, 1997). Lionfish are top/mid level predators in these reef communities and feed on smaller fish and invertebrates (although primarily piscivorous), and have even been observed to cannibalize smaller individuals (Fishelson 1975, 1997, Randall et al. 1990). Like most stationary, reef-dwelling species, lionfish probably feed prolifically at times of abundant food and endure periods of limited feeding when food is scarce, with larger individuals able to withstand longer periods of food deprivation (Fishelson 1997).

Introductions involving other piscivorous predators have been documented in various aquatic ecosystems throughout the world, with most having a deleterious effect on native fishes (Arthington 1991, Fernando 1991, Holcik 1991, Marchetti 1999, Bedarf et al. 2001). Brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) introduced into New Zealand streams have largely outcompeted historically dominant species for food and space resources and nearly decimated several native forage fish stocks (McDowall 2003). Brown trout are thought to be the major cause of declining galaxiid abundance in native streams, while both trout species have been found to consume the entire annual benthic insect production in some New Zealand streams (McDowall 2003). The establishment of Nile perch (*Lates niloticus*) in Lakes Victoria and Kyoga during the late 1950's and early 1960's led to a rapid decline of halochromis cichlid stocks, virtually eliminating the once dominant resident species (Ogutu-Ohwayo and Hecky 1991). Similarly, the competition for food and habitat resources between the introduced redear sunfish (*Lepomis microlophus*) and the native pumpkinseed (*Lepomis gibbosus*) in southern Michigan lakes led to a 56% decline in pumpkinseed abundance (Huckins et al. 2000). Lionfish could have comparable deleterious effects on components of the southeast United States continental shelf ecosystem, but the paucity of information on the biology and ecology of lionfish limits the ability to predict and manage this introduction.

Upon examination of inshore fauna distribution and temperature regimes along the east coast of the United States, Briggs (1974) suggested that Cape Hatteras forms the northern barrier for warm-temperate fauna in the Atlantic Ocean. This faunal separation can be attributed to the effects of temperature acting as both a limiting factor, thus

determining species distribution, and a directive factor, dictating movement patterns (Fry 1971). The abrupt difference in inshore water temperature south and north of Cape Hatteras (Stegmann and Yoder 1996), along with the majority of lionfish observations located south of Cape Hatteras suggests that temperature is an important factor limiting distribution. On the southeast United States continental shelf, lionfish are found in water depth greater than 35 m (Whitfield et al. 2002), while in their native range, lionfish are found in water depths from shore to 50 m (Schultz 1986). The incongruence between the inshore distribution of lionfish in their native range and their primarily offshore distribution in introduced waters could also be a result of temperature-limited distribution. Although temperature seems to be a likely limiting factor in the spread of lionfish along the east coast of North America, no studies have examined the temperature tolerances of lionfish to make predictions.

The present study examined the effects of temperature, rate of temperature decline, and acclimation temperature on the overwintering success and distribution patterns of lionfish on the southeast United States continental shelf. Temperature and oceanographic data for the southeast continental shelf was combined with lionfish low temperature tolerances to create a map of potential habitat in introduced waters. Similar approaches have been used to determine temperature tolerance and predict distribution and overwintering success of red-bellied piranha (*Pygocentrus nattereri*) in the United States (Bennett et al. 1997), southern flounder (*Paralichthys lethostigma*) in Texas estuaries (Prentice 1989), Atlantic croaker (*Micropogonias undulates*) in Mid-Atlantic estuaries (Lankford, Jr. and Targett 2001), and fourteen introduced freshwater fish in Florida (Shafland and Pestrak 1982). A basic understanding of the physiological

requirements of lionfish is a necessary step towards defining the ecological consequences of lionfish to the southeast United States continental shelf ecosystem.

## **MATERIALS AND METHODS**

Two methods are commonly employed to estimate the cold tolerances of fishes: the static method and the critical thermal minimum method (Bennett and Judd 1992). In the static method, a temperature lethal to 50% of fish is estimated by plunging groups of fish from various constant acclimation temperatures into a series of static test temperatures near estimated lower temperature limits (Currie et al. 1998, Beitinger et al. 2000). In the critical thermal minimum method, fish acclimatized to a specific temperature are subjected to a constant linear decrease in temperature until a predefined endpoint is reached (Beitinger et al. 2000). The arithmetic mean of temperatures at this endpoint is termed the critical thermal minimum (CTMin). Traditionally, sublethal endpoints of loss of equilibrium and the onset of muscle spasms were the most widely accepted endpoints. However, researchers in recent years have begun using a modified version of the critical thermal method with death as a valid endpoint (Becker and Genoway 1979, Fields et al. 1987, Prentice 1989, Zale and Gregory 1989, Bennett and Judd 1992, Lankford, Jr. and Targett 2001, Elliott and Klemetsen 2002).

The critical thermal method was utilized for lionfish temperature tolerance experiments with death as a modified endpoint. The critical thermal method allows researchers to simulate natural temperature conditions (Doudoroff 1942, Schwartz 1964, Elliott 1981, Prentice 1989, Baker and Heidinger 1996, Lankford, Jr. and Targett 2001, Hurst and Conover 2002), as well as assess key points such as feeding cessation and behavioral characteristics that may be overlooked using other methods. Since sudden changes of temperature such as those experienced in low thermal plunge conditions are not commonly found in nature (Doudoroff 1942), the critical thermal minimum gives a



more appropriate indication of the survival requirements of fish in natural habitats (Bennett and Judd 1992). Furthermore, this method is economical in terms of test animals, equipment, and time to complete sufficient tests for statistical analysis (Becker and Genoway 1979).

### *General Approach*

To ascertain lionfish temperature tolerance, three separate experiments were conducted. The first experiment examined the effects of winter temperatures commonly observed on the southeast United States continental shelf. Fish were held at a constant acclimation temperature and exposed to a single rate of temperature decline. The effects of faster rates of temperature decline on cold tolerance were examined in the second experiment. All fish were held at a single acclimation temperature and subjected to one of three rates of decline. The third experiment examined the effects of variable acclimation temperatures. Each fish was assigned and held at one of three constant acclimation temperatures, then subjected to a single rate of decline. Upon completion of the experiments, overall CTMin and temperature at feeding cessation values were used in combination with temperature data from the southeast United States continental shelf to predict potential lionfish distribution.

### *Common Methods*

Mean temperatures at which death (CTMin) and feeding cessation occurred were calculated from individual fish observations for each experimental treatment. All fish that did not eat during experimental trials were excluded from mean feeding cessation

calculations. The mean CTMin values and feeding cessation temperatures for each rate and acclimation temperature were compared using Analysis of Variance (ANOVA). Similarly, control group CTMin values were compared with results from the initial experiment and tested for significant differences using ANOVA.

A rate of temperature decline simulating natural conditions was determined from continuous temperature data recorded during 2000, 2001, and 2002 at various locations on the North Carolina shelf. Data were taken from five sites where lionfish were observed: the Naeco-stern, Papoose, Atlas, Normania, and Lobster Wreck, along with one additional site, the Suloid (Figure 1A). Temperatures from each site were plotted against time (Figure 1B) and sharp drops in winter (December, January, February, March) temperatures were chosen for further analysis. The average duration of the events was 4 days. The slopes from each event were averaged (overall mean =  $1^{\circ}\text{C d}^{-1}$ ; Figure 1C) and this rate ( $1^{\circ}\text{C d}^{-1}$ ) was used for all experimental trials and controls, except those examining variable rates of temperature decline.

Juvenile lionfish were purchased from a commercial vendor (The Marine Center, Dallas, Texas) for all three experiments. All lionfish were initially labeled (by the vendor) as *P. volitans*, but meristic analyses later identified some lionfish of Sumatran origin (origin characterized and labeled by the vendor) as *P. miles*. Experiments were conducted independent of species designation. Two facilities were used to house fish prior to experimental trials. Fish housed at the North Carolina Aquarium at Pine Knoll Shores were kept in two 200-gallon holding tanks in a flow-through seawater system under a constant temperature of  $25^{\circ}\text{C}$  and a salinity of 35. Lionfish kept at the NOAA Beaufort Laboratory were held in four holding tanks at  $25^{\circ}\text{C}$  and 35 salinity prior to

experiments. Each holding tank operated as a recirculating seawater system consisting of a 180-gallon semi-square polyethylene tank and an individual biofiltration unit. Seawater for each tank was filtered and adjusted with Instant Ocean and/or distilled water to achieve desired salinity. Approximately 15-20% water changes were made bimonthly, after which salinity was measured with a compensated salinity refractometer and adjusted accordingly. Temperature was measured daily. An approximate photoperiod of 10h light to 14h dark was maintained at each housing facility.

All individual temperature trials were conducted at the NOAA Beaufort Laboratory. Four West Coast Aquatics 50-gallon Mini Ocean Aquaria were used for the experimental tanks, each equipped with recirculation and refrigeration units, biological filtration, and a digital thermometer. Experimental tanks contained distilled water treated with Instant Ocean to achieve desired salinity. Photoperiod was approximately 10h of light to 14h dark and salinity was held at 35 for all trials. Salinity was monitored weekly and adjusted as needed.

Lionfish held at both the Pine Knoll Shores Aquarium and at the NOAA Beaufort Laboratory were initially fed a mixture of frozen and live fish (*Anchoa* spp.), but were later fed a diet of entirely live fish (*Gambusia affinis*, *Leiostomus xanthurus*, *Lagodon rhomboides*, and *Fundulus* spp.) from local waters. Fish were fed to satiation every 2-3 days while in holding tanks, and offered food approximately every 1-2 days while in experimental tanks.

#### *Experiment 1: Effects of North Carolina winter temperatures*

The effects of winter temperature decline were determined from a group of 10 lionfish (Table 1). All fish used for experiment 1 were housed and maintained at the

North Carolina Aquarium at Pine Knoll Shores. Individual fish were transferred from holding tanks to separate experimental tanks and allowed to recover for a period of 24h before experimental trials began. The temperature was decreased at a constant rate of  $1^{\circ}\text{C d}^{-1}$  until death to simulate the average winter temperature rate of decline exhibited at North Carolina sites with lionfish. A calibrated NIST traceable digital thermometer was used to measure temperature at least three times daily. Observations and temperatures were recorded for each fish when feeding cessation, loss of equilibrium, and death occurred. Death was defined as the cessation of fin, body, and opercular movement and a total lack of organismic response (Becker and Genoway 1979, Lankford, Jr. and Targett 2001). Upon death, fish were removed from the tank, weighed (wet weight) and measured (standard length) for later analysis.

#### *Experiment 2: Effects of rate of temperature decline*

To examine the effect of variable rates of temperature decrease on cold tolerance, lionfish were subjected to three rates of decrease:  $3^{\circ}\text{C d}^{-1}$ ,  $2^{\circ}\text{C d}^{-1}$ , and  $1^{\circ}\text{C d}^{-1}$ . For each respective rate of decline, 6, 6, and 3 fish were used (Table 1). These faster rates of decline were chosen to simulate extreme daily temperature drops occasionally observed at sites with lionfish (Figure 1C). All fish used for experiments examining rate of temperature decline were housed at the NOAA Beaufort Laboratory. Individual fish were randomly assigned a rate of decrease and then transferred to an experimental tank. After a 24h recovery period, experiments were conducted following the same procedure outlined above for experiment 1 with rate of decrease adjusted accordingly.

### *Experiment 3: Effects of acclimation temperature*

To assess the effect of acclimation temperature on cold tolerance, lionfish were acclimated at constant temperatures of 15°C, 20°C, and 25°C. For respective acclimation temperatures, 6, 6, and 3 fish were used for experimental trials (Table 1). These temperatures were chosen to represent temperature levels close to thermal limits and commonly experienced during winter months (15°C) as well as levels commonly experienced in spring/fall (20°C) (Figure 1B). Lionfish used for acclimation temperature experiments were housed at the NOAA Beaufort Laboratory. Holding tank temperature of 25°C was maintained using three 300W immersion aquarium-type heaters, while a drop-in titanium coil chiller (Cyclone©) was used to maintain 15°C. Holding tank temperature of 20°C was controlled by ambient air temperature in the laboratory. Water temperatures were decreased 1.0°C d<sup>-1</sup> ( $\pm 0.5^\circ\text{C}$ ) from the 25°C initial holding temperature until constant acclimation temperatures were achieved. All fish were held at final acclimation temperatures for at least 20 days prior to experimental trials. A 24h recovery period was allotted after transfer to individual experimental tanks before each trial was begun. Water temperature was then decreased at a rate of 1°C d<sup>-1</sup> for each trial following the above procedure.

### *Distribution and potential overwintering range*

To estimate the overwintering range and distribution of lionfish on the southeast United States continental shelf, long-term offshore temperature data were analyzed and referenced with hard bottom location data. Three hundred and eighty two sea surface temperature images from NOAA's CoastWatch program for the month of February

(1995-2003) were autogeoreferenced (Ferguson et al., in review) and a composite image was developed from the median pixel values. Median values limited the effect of cloud coverage on the composite image. Minimum temperatures in the inner southeastern continental shelf occur in February (Atkinson 1985). Isotherms representing the CTMin and temperature at feeding cessation were extracted from the composite image and used in ArcView to develop a potential habitat distribution for lionfish.

A potential problem of the use of sea surface temperature is that surface temperature does not always represent bottom temperature. Two approaches were used to address this issue. Bottom temperatures were obtained from temperature data loggers at six North Carolina offshore reef sites (Figure 1A). Data loggers were deployed, maintained, and replaced by divers on each site beginning in 2000. Bottom water temperature was recorded every 30 minutes continuously for the duration of deployment. To obtain sea surface temperatures, images for the period of 4 December 2001 to 28 March 2002 were browsed from the NOAA Beaufort Laboratory CoastWatch webpage (<http://www.ccfhrb.noaa.gov/>). Relatively cloudfree images were chosen for subsequent analyses. Sea surface temperatures were extracted and georeferenced from these relatively cloudfree images for the locations of three bottom temperature data loggers using Windows Image Manager (Kahru 2001). Sea surface temperatures were then compared to concomitant bottom water temperatures for the 2001-2002 winter using Pearson correlation. The significant correlations found between surface and bottom temperatures at the Naeco, Papoose, and Suloid support the use of sea surface temperature images for estimating the overwintering range and distribution of lionfish on the southeast United States continental shelf (Figure 2).

A comparison of isotherms derived from sea surface temperatures with those derived from long-term southeast United States continental shelf bottom temperature data also indicated a high level of agreement between sea surface and bottom temperatures on the southeast continental shelf (Figure 3). Bottom temperature contours were derived from National Oceanographic Data Center profiles ( $n = 5000$ ) for years 1950 to 1999 and organized into a grid of monthly averages for the middle-southern portion of the southeast United States continental shelf (data reported in Blanton et al. in press and provided by Brian Blanton, University of North Carolina at Chapel Hill). Monthly averages were then transformed from ASCII grid to ARC/INFO files, where February 11°C, 13°C, and 16°C bottom temperature isotherms were calculated and imported into ArcView for comparison with sea surface temperature isotherms (see above). The concordant results of these comparisons again support the use of sea surface temperatures as a proxy for bottom water temperatures on the southeast United States continental shelf during winter months.

## RESULTS

### *Experiment 1: Effects of North Carolina winter temperatures*

Lionfish exhibited a highly consistent pattern of behavior when subjected to gradual temperature decrease. After transfer to experimental tanks, all fish were responsive to visual and physical stimuli, had slow opercular ventilation, and explored their surroundings (i.e., normal behavior). At temperatures of 13°C and below, fish were unresponsive to visual stimuli (but still responded to touch), displayed darker coloration, and became stationary and lethargic. Once stationary, all fish positioned dorsal, pectoral, and pelvic fins streamlined against their body and oriented themselves into a corner of the tank for the remainder of the experiment. Temporary loss of equilibrium and swimming bursts were observed at temperatures just prior to death with permanent loss of equilibrium at temperatures at which death occurred. Swimming bursts usually consisted of swimming rapidly for 3-5 seconds (occasionally bumping into tank walls) followed by total cessation of movement, at which point fish sank to the bottom and remained motionless. All fish fed prior to experimental trials. The majority of fish fed at or below the initial temperature setting, with only one fish not eating throughout the entire trial. Fish generally ate less at lower temperatures and no fish were observed feeding below 13°C. The mean CTMin value was 10.7°C with very little variation (SD = 0.483; Figure 4). The mean temperature at feeding cessation was 15.3°C (SD = 2.000; Figure 4).

### *Experiment 2: Effects of rate of temperature decline*

Fish generally followed behavior patterns observed in experiment 1. All fish in the 3°C d<sup>-1</sup> group displayed normal behavior until temperature was decreased to 13°C, at



which point fish became lethargic and stationary. At 10°C temporary loss of equilibrium and swimming bursts began to occur. These changes in behavior were rapidly followed by permanent loss of equilibrium and death for all individuals in this group. Those fish exposed to a 2°C d<sup>-1</sup> rate of decrease behaved similarly. At 13°C the majority of fish became stationary and lethargic. After temperature was decreased to 11°C, all fish experienced temporary loss of equilibrium and swimming bursts. Again, these changes in behavior were followed rapidly by permanent loss of equilibrium and death, with several individuals observed resting upside down on the tank bottom just prior to death. One fish from this group was eliminated from the stated results due to death from causes other than low temperature. Fish from the 1°C d<sup>-1</sup> group behaved similarly to the fish from experiment 1.

All fish used for experiments involving variable rates of temperature decline fed prior to experimental trials. Fish in the 3°C d<sup>-1</sup> group all fed below the initial temperature setting, however, two fish expelled undigested stomach contents at temperatures just prior to death. Two fish in the 2°C d<sup>-1</sup> group did not eat for the duration of the trial. Additionally, one fish in this group also expelled undigested stomach contents just prior to death. All fish in the 1°C d<sup>-1</sup> group fed during experimental trials and exhibited feeding behavior similar to fish in experiment 1.

Temperature decline rate had no effect on lionfish thermal tolerance. The mean CTMin values for the 3°C d<sup>-1</sup>, 2°C d<sup>-1</sup>, and 1°C d<sup>-1</sup> were 10.0°C (SD = 0.000, n = 6), 10.6°C (SD = 0.894, n = 5), and 9.7°C (SD = 0.577, n=3) respectively (Figure 5). No significant effect of temperature decline rate was found on CTMin (ANOVA,  $p = 0.117$ ; Table 2). For the 3°C d<sup>-1</sup>, 2°C d<sup>-1</sup>, and 1°C d<sup>-1</sup> groups, feeding cessation occurred at a

mean temperature of 18.0°C (SD = 1.549, n = 6), 17.0°C (SD = 2.000, n = 3), and 15.3°C (SD = 1.528, n = 3; Figure 5). Similarly, no significant effect of temperature decrease was found on the temperature at feeding cessation (ANOVA,  $p = 0.128$ ; Table 2).

### *Experiment 3: Effects of acclimation temperature*

Lionfish exhibited increased activity and feeding behavior at warmer acclimation temperatures during the holding period prior to experimental trials. Fish acclimated to 25°C displayed ‘normal’ behavior (as described in experiment 1) and fed regularly when food was offered. ‘Normal’ behavior was seen in fish acclimated to 20°C, but reduced feeding was observed when fish were offered food. Lionfish acclimated to 15°C were predominantly stationary throughout the entire holding period and fed infrequently, often giving up on catching food after a single attempt. All fish at each acclimation temperature fed during the holding period.

During experimental trials, fish acclimated to 25°C, 20°C and 15°C displayed behavior patterns identical to those observed in experiment 1 as temperature was decreased. All fish in the 25°C acclimation temperature group fed during experimental trials. Five out of six fish fed in the 20°C acclimation group, while only one fish acclimated to 15°C fed during the experimental trial. Several fish in the 15°C acclimation group showed interest and attempted to feed, but quickly abandoned any offered food after an initial unsuccessful attempt.

Acclimation temperature was found to have no effect on low temperature tolerance in lionfish. Mean CTMin values of 9.33°C (SD = 0.335, n = 3), 9.167°C (SD = 0.983, n = 6) and 9.333°C (SD = 0.516, n = 6) were determined for the 25°C, 20°C and

15°C acclimation groups (Figure 6). Acclimation temperature did not have a significant effect on CTMin values (ANOVA,  $p = 0.917$ ; Table 3). Feeding cessation occurred at 14.0°C (SD = 0.000,  $n=3$ ), 15.4°C (SD = 1.517,  $n = 5$ ) and 15.0°C (SD = 0.000,  $n = 1$ ) for fish held at acclimation temperatures of 25°C, 20°C and 15°C (Figure 6). No significant effect was found of acclimation temperature on temperature at feeding cessation (ANOVA,  $p = 0.364$ ; Table 3).

#### *Distribution and potential overwintering range*

An integration of winter isotherms with the experimental results demonstrate that the inshore and northern distribution of lionfish is limited by temperature in southeast United States waters. The application of lionfish low temperature tolerance values in the form of the 11°C and 13°C isotherms constitute the inshore limit on the southeastern continental shelf. With a maximum CTMin value from all three experiments of 11°C (mean = 9.949°C, SD = 0.857), the farthest inshore lionfish are capable of tolerating is predicted to be 11°C. Successful inhabitation is predicted offshore of the 13°C isotherm, due to a minimum temperature of feeding cessation of 13°C (mean = 16.065°C, SD = 2.144) in all laboratory experiments. Using the continental shelf break (200 meter isobath) as the offshore limit along the Atlantic and Gulf coasts, the potential range for lionfish in introduced waters is predicted to extend south from Cape Hatteras onto the shelf region of the Gulf of Mexico (Figure 7). Lionfish sighting locations reported since 2000 agree with the predicted potential range boundaries except for one fish, which was reported from onshore of the 13°C isotherm but offshore of the 11°C isotherm (Figure 8).

It is important to note that while this study indicates that the northern and inshore distribution of lionfish is limited by temperature, the specific boundaries of this potential range will fluctuate over time. Interannual and larger time scale temperature variations as well as alternate data sources will produce varying range limits for lionfish on the southeast United States continental shelf. The addition of future lionfish research also has the capacity to alter current predicted range limits. The limits displayed here were derived solely from data and observations documented in this study and represent the potential range of lionfish in introduced waters based on the best available data.

## DISCUSSION

The laboratory derived temperature tolerance data indicates that lionfish are capable of overwintering on the southeast United States continental shelf. Winter water temperature on the continental shelf is expected to limit inshore distribution and set the northern range limit for lionfish. The current southern limit of the invasion is not bound by temperature, as lionfish could survive but have not yet been reported on the Florida coast south of Miami. These results support the hypothesis that temperature is an important factor limiting survival and dispersal of lionfish in introduced waters (Whitfield et al. 2002), but also indicate that factors other than temperature are limiting the southern extent of this introduction. Experiments on average winter water temperature decline and more extreme thermal events (e.g.,  $3^{\circ}\text{C d}^{-1}$  rate,  $15^{\circ}\text{C}$  acclimation temperature; Figure 1B, 1C) experienced prior to and during winter months indicate  $11^{\circ}\text{C}$  as the inshore limit lionfish can tolerate. Successful inhabitation is predicted offshore of the  $13^{\circ}\text{C}$  isotherm. Although lionfish can tolerate temperatures lower than  $13^{\circ}\text{C}$ , lower temperatures may limit overwintering by controlling feeding behavior (Bennett et al. 1997). Colonization inshore of the  $13^{\circ}\text{C}$  isotherm could occur during summer months, but overwinter mortality related to cold temperatures would prohibit the establishment of persistent populations (Bennett et al. 1997).

Lethal temperature has long been thought to have limiting effects on the geographic distribution and movement of aquatic species (Brett 1956). Studies on low temperature tolerance have primarily been undertaken to predict whether or not a species is capable of overwintering and are especially important when determining the potential range of invasive species (Beitinger et al. 2000). However, information on the cold

tolerance of tropical fishes is lacking. The majority of temperature tolerance data have been generated for temperate fish species (Brett 1956, Elliot 1981, Bennett and Judd 1992, Beitinger et al. 2000). The fact that cold temperatures are not an obvious threat to fish survival in tropical waters (Bennett and Judd 1992), coupled with historically conservative estimates of the general thermal limits of tropical fauna (e.g., 20°C limit, Briggs 1974) may have limited interest in examining thermal tolerances in tropical fish. Yet, with the expansion of tropical species into temperate waters (Parker and Dixon 1998) and the increasing number of marine invasions (Baltz 1991), information on the thermal tolerances of tropical fauna is vital.

Only recently have the effects of low temperature been studied in tropical fish. An examination of cold tolerance of 15 reef species from the eastern tropical Pacific Ocean by Mora and Ospina (2002) determined CTMin values ranging from 10.8°C to 16.3°C. While not exposed to temperatures as cold as those experienced on the southeast United States continental shelf, fish in the Mora and Ospina (2002) study were confronted with cold-water La Nina events sometimes 10°C lower than the normal temperature range of 25°C to 27°C. Bennett et al. (1997) determined that introduced red-bellied piranha were capable of overwintering and likely persisting in the lower southern regions of California, Texas, and Florida. In this case, overwintering range was predicted to better understand red-bellied piranha invasion capabilities and thus allow legislators to enact effective trade regulation policies (Bennett et al. 1997).

An important factor affecting the low temperature tolerance of fishes is rate of temperature change (Beitinger and McCauley 1990). Many researchers have concluded that the rate of temperature change must be slow enough so that the fish's core

temperature does not significantly lag behind water temperature, and rapid enough so test fish do not have time to thermally reacclimate during a trial (Beitinger et al. 2000). However, this produces artificially high rates of change that are often not found naturally. Therefore rates of temperature decline used in this study were chosen to mimic natural temperature regimes, thus increasing the ecological relevance and applicability of the observed results.

Rate of temperature decline was found to have no effect on lionfish temperature tolerance. Doubling and tripling the decline rate had little effect on lionfish CTMin values. Similar results were observed with Atlantic croaker, where a 5-fold increase in rate of decline ( $0.2^{\circ}\text{C d}^{-1}$  to  $1.0^{\circ}\text{C d}^{-1}$ ) did not affect survival at low temperatures (Lankford, Jr., and Targett 2001). Bennett and Judd (1992) found no significant differences in pinfish CTMin values when rate of temperature decrease was tripled ( $0.5^{\circ}\text{C h}^{-1}$  to  $1.5^{\circ}\text{C h}^{-1}$ ). Rate was also reported to have no effect on low temperature tolerance of American shad (*Alosa sapidissima*) exposed to decline rates 4 times higher ( $0.5^{\circ}\text{C d}^{-1}$  to  $1.9^{\circ}\text{C d}^{-1}$ ; Chittenden, Jr. 1972).

Acclimation temperature is thought to be the most important factor influencing thermal tolerance in fish (Lutterschmidt and Hutchison 1997). A review of previous studies by Beitinger et al. (2000) concluded that lethal thermal limits were strongly affected by thermal history immediately prior to experimentation. It is generally thought for low temperature tolerance studies that as acclimation temperature is increased, CTMin is also increased (Beitinger and Bennett 2000). This relationship was supported in studies on largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus*

*punctatus*), rainbow trout (Currie et al. 1998), and red drum (*Sciaenops ocellatus*) (Ward et al. 1993).

Contrary to these studies, low temperature tolerance of lionfish was unaffected by acclimation temperature. Differences of 10°C between acclimation temperatures produced no significant differences in CTMin. Brett (1956) reported that gain in resistance to low temperatures (i.e., acclimation) is a slow process, requiring some species up to 20 days to fully acclimate. Lionfish were left at acclimation temperatures for at least 20 days (some greater than 40 days) prior to experimental trials. Similarly, acclimation history had no influence on the lower thermal limit of splittail (*Pogonichthys macrolepidotus*) acclimated to temperatures ranging from 12°C to 20°C (Young and Cech, Jr. 1996). Lionfish insensitivity to acclimation temperature and rate of decline could be indicative of different temperature tolerance relationships for tropical species (Beitinger and Bennett 2000).

The application of thermal limits to oceanographic and hydrographic characteristics of the Atlantic coast of the United States defines the northern and inshore limit of lionfish distribution. The eastern United States continental shelf is divided into two zoogeographic regions. The southeast continental shelf ranges from Florida to Cape Hatteras and is characterized by tropical and warm temperate fish fauna (Briggs 1974). Winter water temperatures between 12°C and 16°C and salinities of 34 to 36 are common on the southeast shelf (Atkinson 1985, Whitfield et al. 2002). The northeast continental shelf spans from Cape Hatteras north to the Gulf of Maine and consists of colder (5°C to 10°C winter temperature range), and less saline (30 to 33 salinity) waters with a cold temperate fish fauna (Briggs 1974, Mountain and Holzwarth 1989, Whitfield et al. 2002).



The Gulf Stream flows along the southeast continental shelf, separating from the shelf and turning seaward at Cape Hatteras, the geographic divide between the southeast and northeast United States continental shelf (Briggs 1974). The absence of the Gulf Stream along the edge of the northeast continental shelf coincides with cooler Labrador Current water flowing south and forming the cold temperate water mass characteristic of the northeast shelf. During winter, the water of the northeast shelf is too cold for lionfish survival and thus, Cape Hatteras marks the northern range limit along the Atlantic coast.

The occurrence of all reported adult lionfish sightings off the coasts of North Carolina, South Carolina, Georgia, and Florida supports Cape Hatteras as the northern range limit of lionfish along the east coast of North America (Figure 8). Juvenile lionfish have been observed on the northeast United States continental shelf during summer, but these fish could not survive winter temperatures. McBride and Able (1998) reported a similar fate for spotfin butterflyfish (*Chaetodon ocellatus*) in New Jersey estuaries during winter, where juveniles were found to stop eating at 12°C and perish at temperatures below 10°C. Like lionfish, adult spotfin butterflyfish are commonly found in warm temperate and tropical waters (Robins et al. 1986). Juveniles of both species likely occur on the northeast United States continental shelf as a result of larval transport from the southeast to northeast continental shelf (Hare and Cowen 1996, Hare et al., 2002). For both the lionfish and the spotfin butterflyfish, larval transport supplies juveniles to appropriate habitat in the northeast United States continental shelf ecosystem, but winter cooling causes overwinter mortality (McBride and Able 1998).

Water temperature also limits the inshore distribution of lionfish on the southeast United States continental shelf. Heat is constantly supplied to the edge of the southeast

United States shelf owing to the Gulf Stream at the shelf edge (Barnard et al. 1997). During the winter, heat is lost across the entire shelf resulting from air-sea interactions (Atkinson 1985). The supply of heat from shelf edge with the loss of heat across shelf during winter creates a cross-shelf gradient in shelf temperatures (Figure 3). Owing to the thermal tolerance of lionfish, the inshore portions of the southeast shelf are too cold for overwinter survival. Conversely, the heat supplied from the Gulf Stream, creates thermally appropriate overwintering habitat on the outer portion of the shelf. This overwinter habitat extends closer to shore southward from Cape Hatteras to southeast Florida, as the shelf narrows and winter heat loss to the atmosphere decreases. As a result, lionfish can survive across the entire shelf off of Florida, but only on the outer half of the shelf off of North Carolina (Figure 8).

The offshore and southern limits of the lionfish invasion are not linked to temperature tolerances. In their native range, lionfish are reported from 0-50 m (Schultz 1986). Along the southeast United States continental shelf, lionfish have been observed as deep as 79-80 m (Steve Ross, pers. comm.), with the majority observed between 35-45 m (Whitfield et al. 2002). The specific factors determining depth limits of lionfish have not been quantified, however, lionfish distribution on the southeast continental shelf is generally limited offshore by the shelf break. The shelf break typically occurs at 75 m on the southeast continental shelf, however, since lionfish were observed offshore of 75 m and there is little difference between the 75 m and 200 m isobaths (Lee et al. 1985), 200 m was chosen to represent the shelf break in this study. Furthermore, the 200 m isobath coincides with the 13°C bottom temperature isotherm (data reported in Blanton et al. in press and provided by Brian Blanton, University of North Carolina at Chapel Hill) during

winter months on the southeast United States continental shelf, but future work is necessary to determine the precise mechanism of lionfish offshore limitation.

Based on distribution in their native range and the thermal tolerances reported here, lionfish have a potential distribution in the Atlantic Ocean spanning as far south as southern Brazil. The native range of lionfish extends nearly 70° of latitude (roughly 35°S to 35°N) at both its western and eastern bounds. Lionfish are distributed from the southeast Mediterranean Sea (Golani and Sonin 1992) and Red Sea to South Africa, and in the Indo-West Pacific from southern Japan, south to Lord Howe Island off the east coast of Australia (Schultz 1986, Whitfield et al. 2002). In the southwest Pacific Ocean, the flow of the warm East Australian Current along northeastern Australia and through the Tasman Sea permits the inhabitation of tropical and subtropical fauna as far south as Lord Howe Island (Briggs 1974). Similarly, the warm water of the southward Agulhas current is responsible for maintaining a warm temperate fauna on the southeast coast of Africa and its retroflexion east as it nears the Cape of Good Hope serves as a faunal changing point (Briggs 1974). The application of the southern native range limits to the western Atlantic Ocean indicates lionfish could potentially be distributed in the Gulf of Mexico, throughout the Caribbean Sea and southward along the coast of Brazil. The confluence of the Brazil Current and the Falkland Current near the mouth of the Rio de la Plata redirects the Brazil Current eastward and thus designates the boundary for warm temperate fauna in the southwestern Atlantic Ocean (Briggs 1974) and marks the potential southern limit for lionfish in introduced waters.

However, despite a potential Atlantic distribution extending into the southern hemisphere, lionfish distribution has been limited to the southeast United States

continental shelf north of Miami. This southern limit is likely artificially constrained by the site of initial lionfish introduction. The accidental or intentional release of lionfish from aquaria into Biscayne Bay, Florida (Courtenay 1995, Whitfield et al. 2002) exposed introduced lionfish to Atlantic waters at the convergence point of the Florida Current with the Gulf Stream (Leipper 1954). This combined northward flow would result in larval and juvenile transport along the Atlantic coast via the Gulf Stream, explaining the present dispersal of lionfish on the southeast continental shelf (Figure 8). However, no sufficient countercurrents are present in the vicinity of Biscayne Bay to allow for transport southward. Therefore an introduction site along the Florida Keys, Gulf coast, Caribbean Sea, or Brazilian coast would be necessary for the establishment of lionfish south of Florida.

Several characteristics of lionfish support both the current and potential range in southeast United States waters. Lionfish are particularly susceptible to the effects of low temperatures (in terms of feeding, growth, and activity). Also, like many reef fish, they are sedentary after settlement and exhibit high site fidelity. The latter two characteristics generally render reef fish unable to move to areas with better physical conditions, as is commonly seen with pelagic species experiencing unfavorable conditions (McBride and Able 1998, Mora and Ospina 2002). For these reasons, any lionfish inhabiting reefs inshore of the 13°C winter isotherm or above Cape Hatteras during summer would most likely be unable to overwinter and perish. These same characteristics would be expected to limit and direct distribution if lionfish were introduced outside of the southeast United States continental shelf ecosystem.

The introduction of lionfish along the Atlantic coast of the United States illustrates several important factors concerning marine invasions. Characteristics of fish in their native range cannot always be directly applied to introduced habitats and are often a poor predictor of performance in a novel setting (Ruiz et al. 1997). For example, while the depth range of lionfish is from shore to 50 m in the Indo-Pacific, the majority of lionfish have been observed at depths greater than 35 m along the southeast coast of the United States. The lionfish introduction also exhibits how fish can be initially introduced by human-mediated means, then subsequently transported by natural dispersal mechanisms. Fish introduced into coastal ecosystems are most often subjected to current systems, placing a major emphasis on both the physical release site and timing of such an event. Additionally, the establishment of lionfish along the Atlantic coast demonstrates the susceptibility of overfished, species-depleted habitat to invasion. Successful establishment most often occurs in environments with benign temperature regimes, disturbed or altered habitats, or those where native assemblages have been temporarily disturbed or depleted (Moyle 1985, Baltz 1991, Moyle and Light 1996). The persistent and intense fishing pressure of the United States commercial fishing industry has produced the requisite depauperate fish communities that have likely aided the invasive success of lionfish along the Atlantic coast of the United States.

Table 1. Number of lionfish, rate of temperature decline, and acclimation temperature ( $T_{ACCL}$ ) used in each experiment.

Treatment	$T_{ACCL}$ Rate	25			20	15
		1	2	3	1	1
Exp 1	Winter Temperature Decline Rate	10				
Exp 2	Variable Rate of Temperature Decline	3	6	6		
Exp 3	Variable Acclimation Temperature	3			6	6

Table 2. Results of ANOVA examining the effect of rate of temperature decline on CTMin and temperature at feeding cessation in Experiment 2.

Source	Sum of squares	df	Mean square	<i>F</i>	<i>p</i>
Rate (CTMin)	1.848	2	0.924	2.628	0.117
Error	3.867	11	0.352		
Rate (Feeding)	14.250	2	7.125	2.600	0.128
Error	24.667	9	2.741		

Table 3. Results of ANOVA examining the effect of acclimation temperature ( $T_{ACCL}$ ) on CTMin and temperature at feeding cessation in Experiment 3.

Source	Sum of squares	df	Mean square	$F$	$p$
$T_{ACCL}$ (CTMin)	1.000	2	0.050	0.088	0.917
Error	6.833	12	0.569		
$T_{ACCL}$ (Feeding)	3.689	2	1.844	1.203	0.364
Error	9.200	6	1.533		



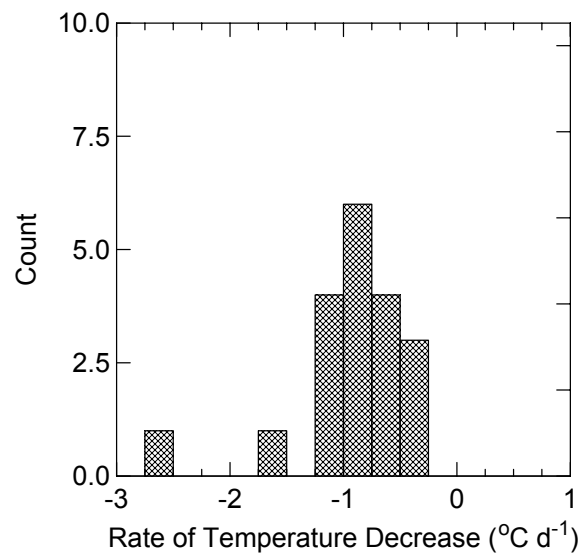
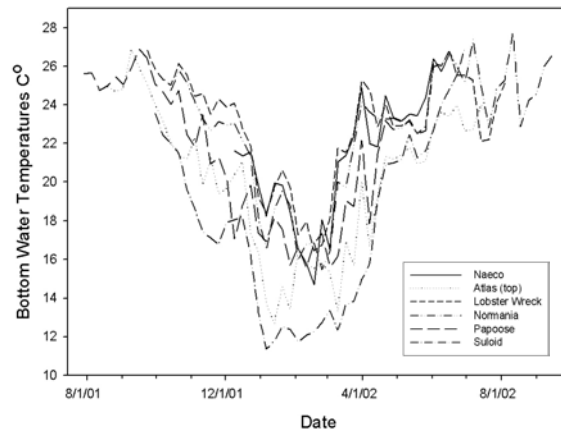
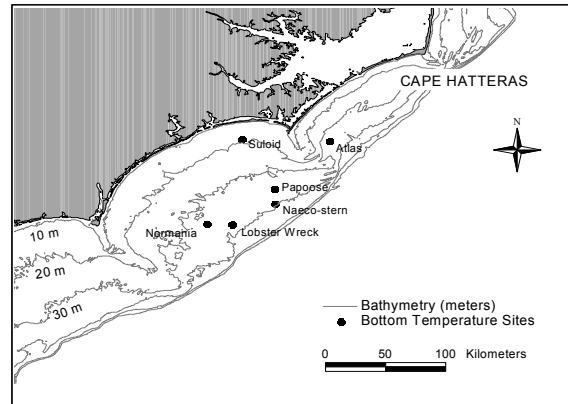


Figure 1. (A) Location of temperature loggers along North Carolina coast with bathymetry shown to 60 meters, (B) bottom temperatures recorded at each site in winter 2001-2002, (C) rate of temperature decline per day for 19 cold events recorded from sites shown in A.

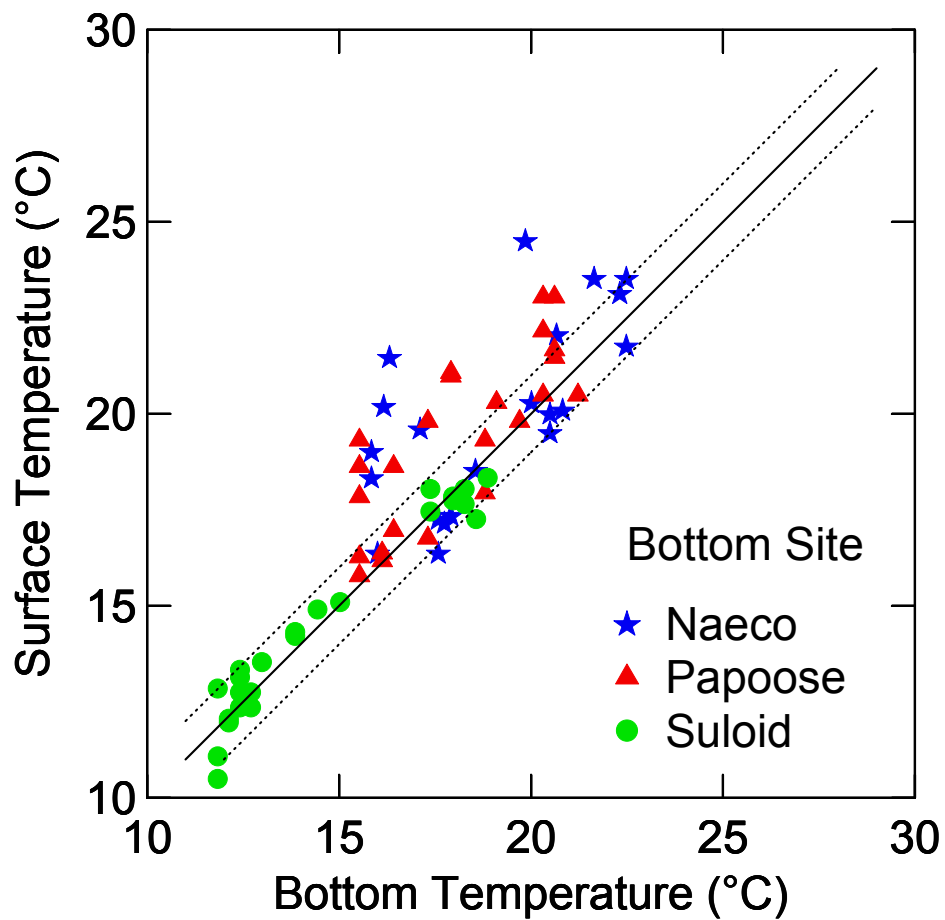


Figure 2. Correlation between satellite-derived sea surface temperature and bottom temperature at three North Carolina bottom sites: the Naeco ( $r = 0.671$ ), Papoose ( $r = 0.796$ ), and Suloid ( $r = 0.973$ ). A 1:1 relationship is shown along with lines depicting  $\pm 1^\circ\text{C}$  for reference.

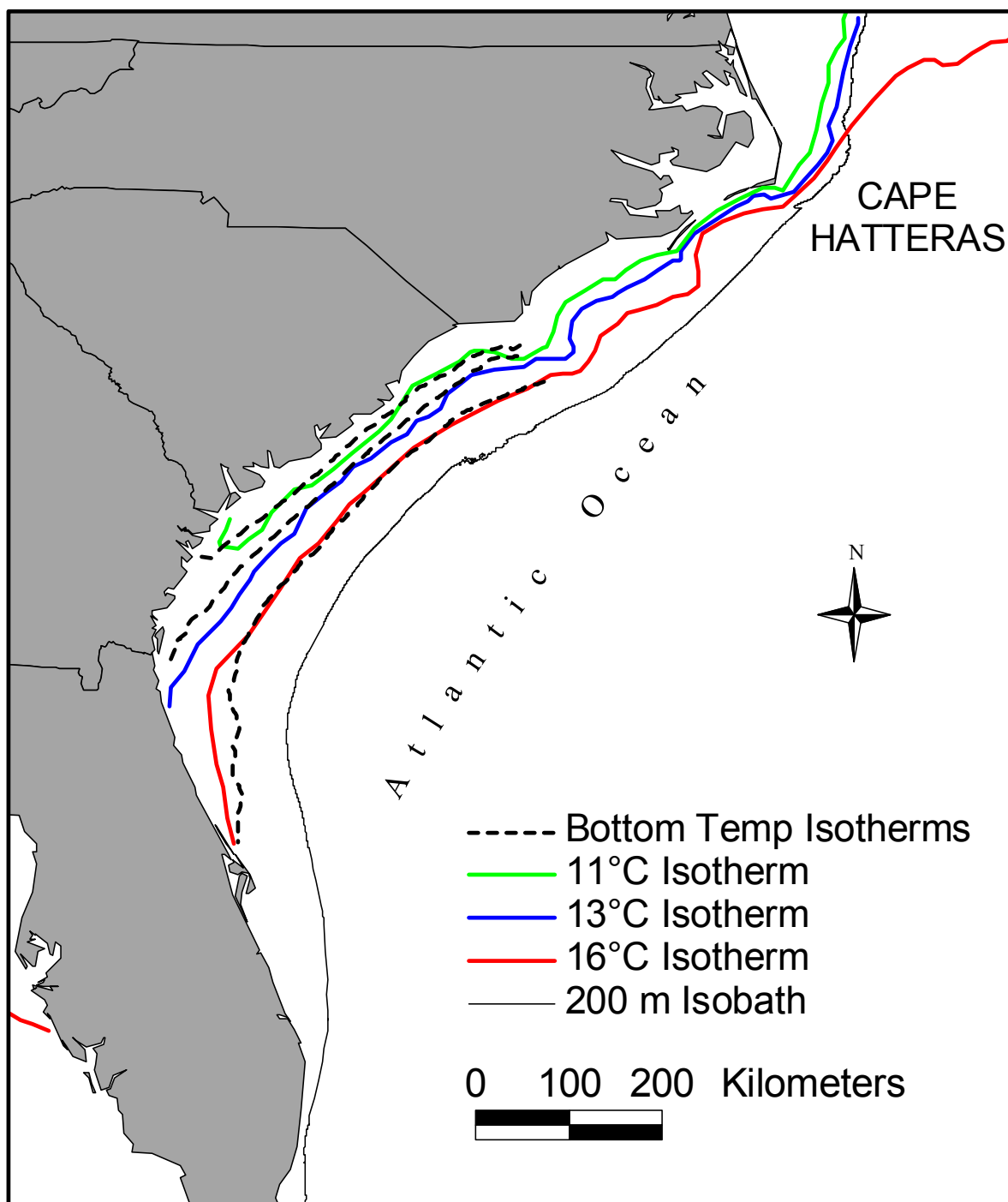


Figure 3. February bottom temperature isotherms derived from 1950-1999 climatology of the southeast United States continental shelf (from Blanton et al. in press) and satellite-derived surface temperature isotherms developed as part of this study.

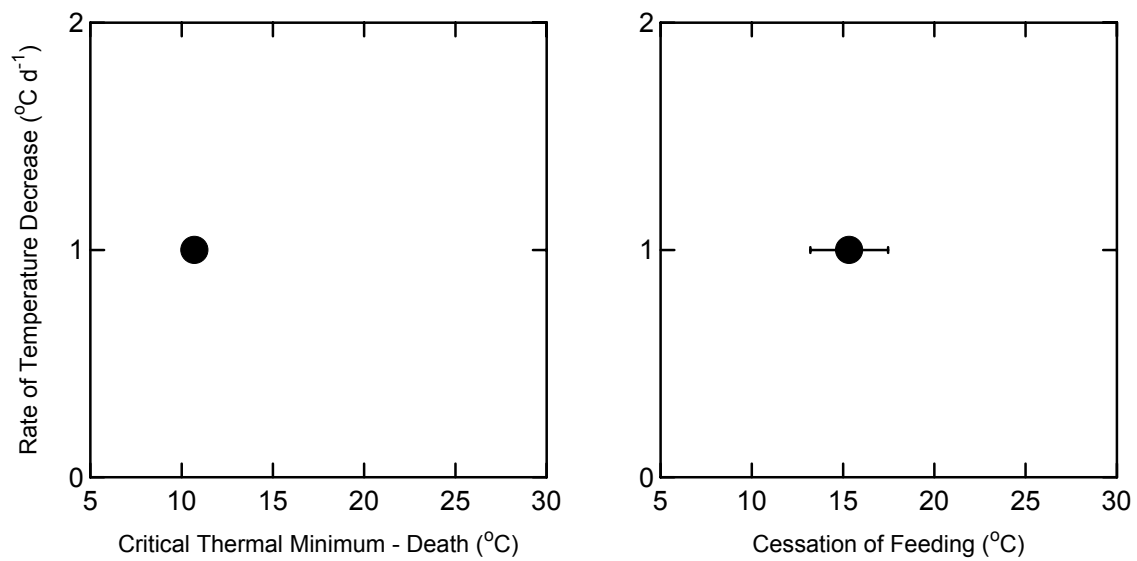


Figure 4. Critical thermal minimum and average temperature at feeding cessation observed at initial rate of temperature decrease (Exp 1). Standard error bars are shown.

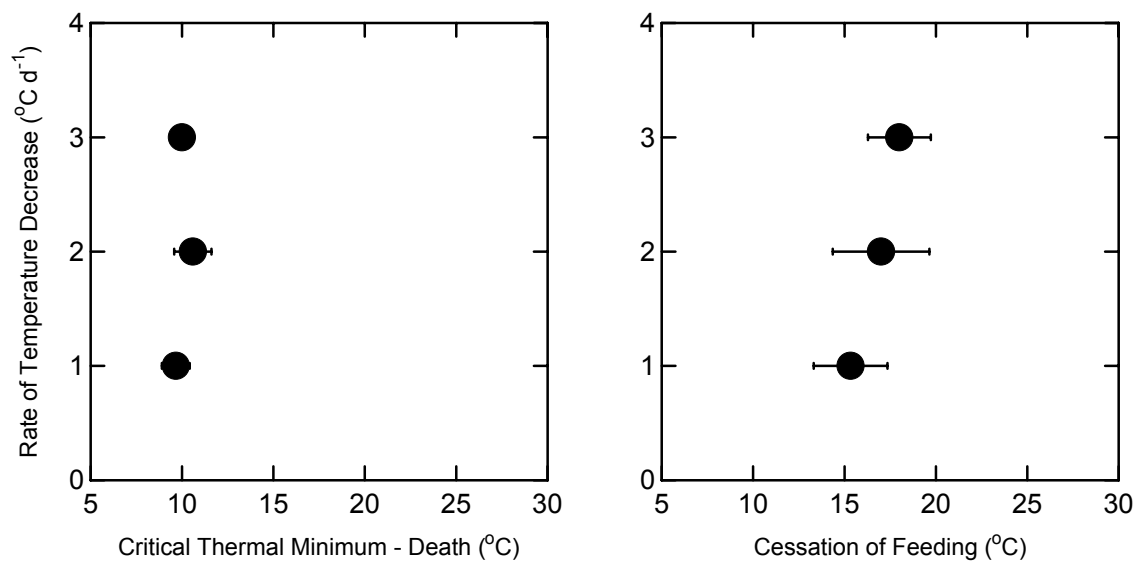


Figure 5. Critical thermal minimum and average temperature at feeding cessation observed at three rates of temperature decrease (Exp 2). Standard error bars are shown.

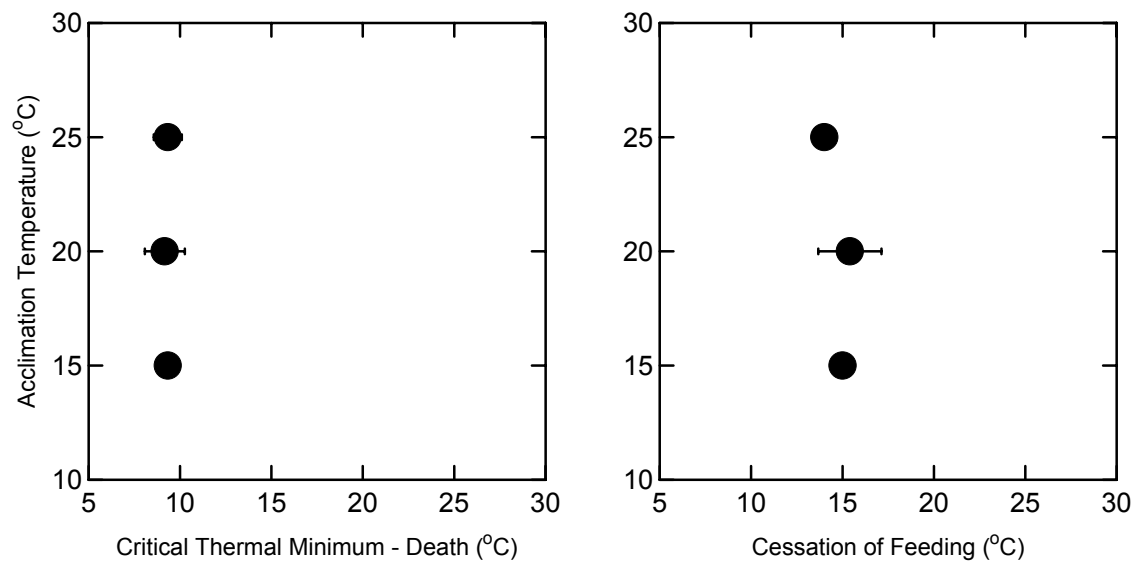


Figure 6. Critical thermal minimum and average temperature at feeding cessation observed at three acclimation temperatures (Exp 3). Standard error bars are shown.

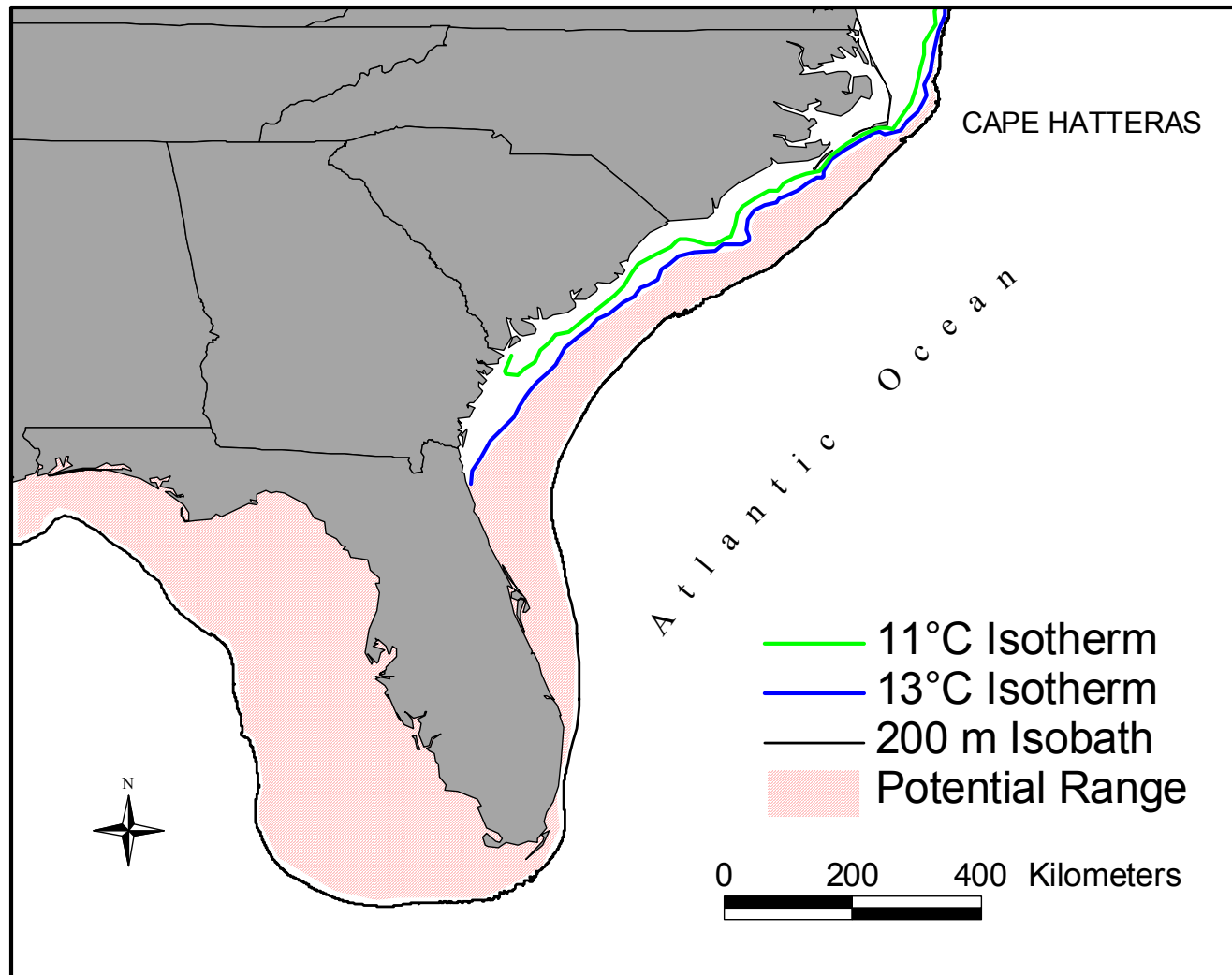


Figure 7. Potential range for lionfish on the southeast United States continental shelf predicted from 13°C isotherm. Offshore limit is 200 m isobath.

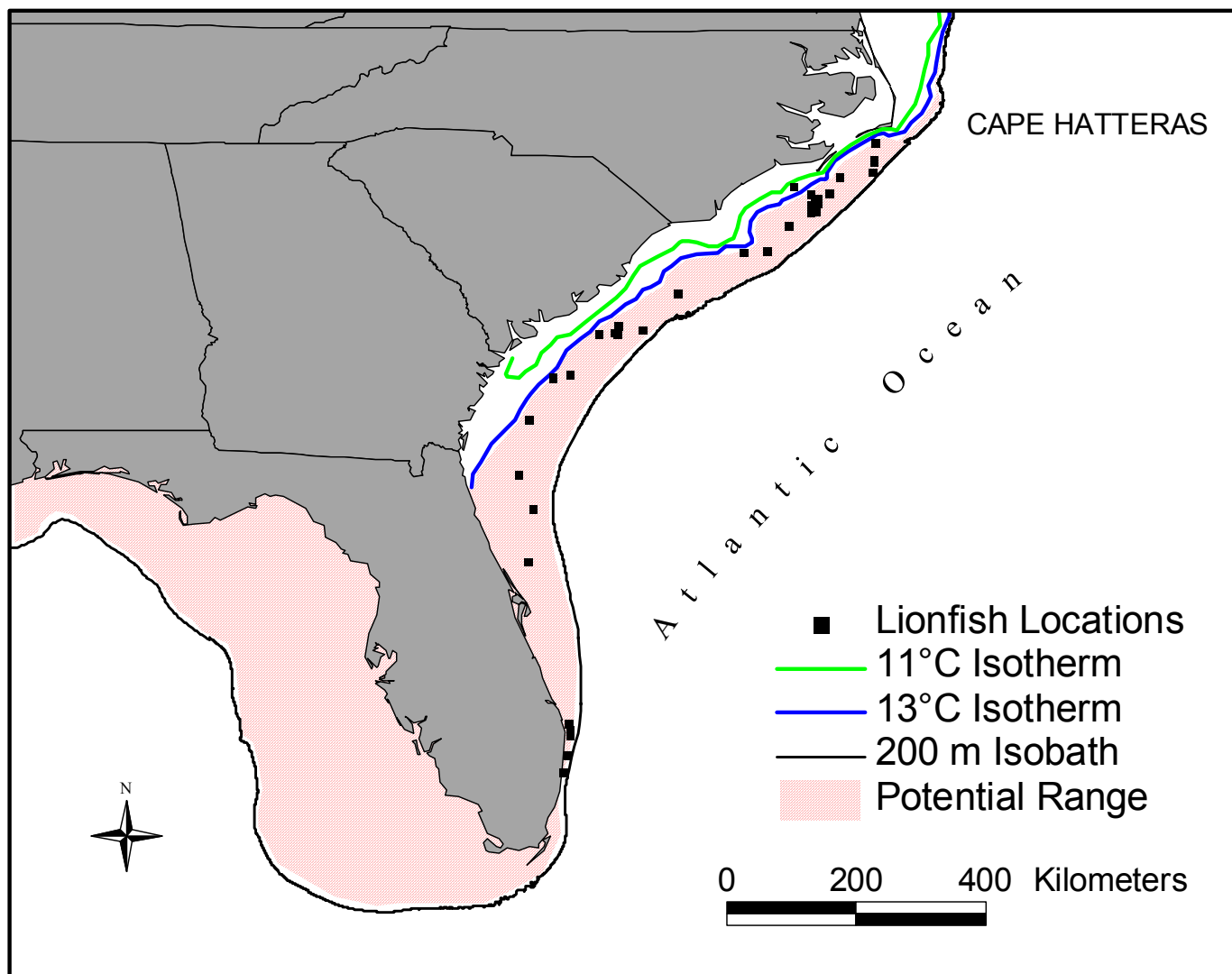


Figure 8. Location of lionfish sightings since 2000. (Lionfish location data courtesy of Paula Whitfield, NOAA Beaufort Lab).



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