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Thermal Preferences and Critical Temperature Regimes of the Western North Atlantic Invasive Lionfish Complex (Pteroist spp.)

Benjamin Barker
Nova Southeastern University, barker.ben294@gmail.com

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Thermal preferences and critical temperature regimes of the western North Atlantic invasive lionfish complex (*Pterois* spp.)

By
Benjamin D. Barker

Submitted to the Faculty of
Nova Southeastern University
Halmos College of Natural Sciences and Oceanography
in partial fulfillment for the requirements for
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Benjamin D. Barker

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Approved:
Thesis Committee

Major Professor: ____________________________
David W. Kerstetter, Ph.D.

Committee Member: __________________________
Andrij Z. Horodysky, Ph.D.
Department of Marine Science and Environmental Science
Hampton University

Committee Member: __________________________
James D. Thomas, Ph.D.
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Abstract

Temperature preference, behavioral tolerance, and physiological tolerances were determined for locally captured, invasive juvenile lionfish at four different acclimation temperatures (13°C, 20°C, 25 °C and 32°C). Temperature preferences and avoidance temperatures were evaluated using an automated shuttlebox system that presents subject-driven temperature stimuli to subjects, who control the temperature with their movement throughout the tank for 12 hours. Subjects are tracked by a computer system, with data output approximately every second. Acute preference was calculated from the archived data as the mean temperature that the fish occupied during the first two hours of dynamic experimentation. Acute preference measurements were used to determine final temperature preferendum and avoidance temperatures were used to determine behavioral tolerance. Critical thermal methodology (CTM) determined the CT_min and CT_max of the lionfish with loss of equilibrium (LOE) as the endpoint. It is assumed that beyond this temperature, the fish would be unable to survive. Temperature was increased or decreased by 0.33°C per minute until the end point was reached. Thermal tolerance polygons provide a visual representation of the lower and upper thermal avoidance temperatures, delineating the thermal range of the species. Their CT_min and CT_max (acclimated to 25°C) were compared experimentally with two other Florida reef fish species (Cephalopholis cruentata and Lutjanus apodus). Acute preferences of juvenile invasive lionfish showed a final preferendum at 28.7 ± 1°C, but with no significant difference between acclimation temperatures. The thermal tolerance polygon of invasive lionfish shows a strong correlation between CTM and acclimation temperature, with the highest CT_max at 39.5°C and the lowest CT_min at 9.5°C. The thermal polygon, preference, and avoidance data describes the thermal niche of the lionfish. Lionfish CTM (24.61°C) is narrower than those of C. cruentata (25.25°C) and L. apodus (26.87°C).

Keywords: Pterois volitans, lionfish, invasive, preference, thermal tolerance, shuttlebox, CTM
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Introduction

Humans have been responsible for the introduction of many exotic species into new areas. Many of these introductions will go unnoticed, but some have the potential to impose direct impacts on the existing ecosystems, human health, or human activity. Exotic species may thrive when introduced because their fitness is increased in new areas compared with their native habitat, potentially due to reduced predation, parasitism, competition, and/or increased food abundance (Kimbro et al. 2009; Mooney and Cleland 2001; Torchin et al. 2003). Unfortunately, exotic species can have an adverse affect on the existing ecosystem by outcompeting native species. If an exotic species threatens the native ecosystem, it is then considered invasive. Understanding invasive species is the first step in minimizing undesirable impacts.

Invasive species can change how existing ecosystems are structured and often negatively affect commercial, agricultural or recreational activities in the process (Pimentel et al. 2005). One example is the introduction of lionfish Pterois spp. into tropical Atlantic waters. Since their establishment, lionfish have changed the structure of the tropical Atlantic reef ecosystem, impacted commercial and recreational fisheries, and have received a great deal of attention from scientists, managers, and the general public (Morris and Whitfield 2009). The invasive lionfish is quickly becoming one of the most impactful marine invasive species to date and will continue to affect the tropical Atlantic marine ecosystem (Morris and Whitfield 2009).

Background

The phylogeny of the invasive lionfish complex in the Atlantic and Caribbean remains uncertain. Most literature describes the invasive lionfish complex in the Atlantic and Caribbean to be composed of two species, Pterois volitans [Linnaeus 1758; “red lionfish”] and Pterois miles [Bennett 1828; “common lionfish”] (Hamner et al. 2007; Kochzius et al. 2003). However, recent studies have shown that P. volitans may instead be a hybrid of other Pterois species (specifically, P. miles and P. lunulata/russelii), rather than a distinct species (Wilcox 2014). Species of Pterois are members of the family Scorpaenidae, which includes other species of lionfish, stonefish, and scorpionfish. The
maximum size of the invasive lionfish within the literature is 48-49 cm; however, individuals reach sexual maturity at approximately 10 cm (Darling et al. 2011; Jud and Layman 2012). Invasive lionfish in the western North Atlantic are also much larger compared to individuals from their native ranges (Darling et al. 2011), which is known to also occur in other invasive species (e.g. invasive plants; Blossey and Notzold 1995). Invasive lionfish coloration is typically alternating white and reddish vertical bands on the body with spots on their soft fins. They have 13 dorsal spines, 2-4 pelvic spines, and 2-3 anal spines, all containing an acetylcholine-based venom. Lionfish also display a number of soft fin rays, including their fan-like pectoral fins. Their heads are usually covered with soft tentacles (cirri), which change shape and size throughout their life.

Invasive lionfish are highly adaptable, since they inhabit many different habitats, utilize different feeding strategies, and prey upon a wide range of fish and invertebrates. Invasive lionfish have been documented not just on coral reefs, but also in nearly all habitats that provide structure. This list includes artificial habitats, deep habitats, mangroves, sea grasses, and estuaries (Barbour et al. 2010; Jud et al. 2011). Lionfish are known to use multiple feeding strategies. They can ambush, stalk, and corner their prey with their large pectoral fins, or they can stir up sand and capture fleeting invertebrates (Morris and Akins 2009). Although lionfish are mainly piscivorous as adults, they do consume more crustaceans as juveniles, suggesting an ontogenetic diet shift occurring over their life span (Dahl and Patterson 2014; Jasper 2013). Feeding usually occurs during the crepuscular times when they are typically more active, but they have been shown to eat mid-day as well (Morris and Akins 2009). Predation upon lionfish is mostly unknown in both their native and invasive range. However, there are reports of the Pacific cornetfish *Fistularia commersoni* feeding on lionfish in their native habitat (Robins 2014). Lionfish have also been found in the stomachs of grouper species in their invasive range (Robins 2014).

**Invasive Lionfish**

Confirming the mechanism for introduction of invasive lionfish into North Atlantic waters is nearly impossible. However, deliberate aquarium release is widely considered the most likely mode due to the similarity between invasive specimens and
lionfish from Indonesia and the Philippines (Hare and Whitfield 2003), a region from which roughly 85% of marine aquarium fishes imported into the United States are collected (Whitfield et al. 2002). The first sighting of lionfish in the wild within the western North Atlantic occurred in 1985, when one individual was captured in a lobster trap off of Dania Beach, Florida (Schofield 2009). Subsequent reported lionfish sightings were relatively scarce until 2000, when lionfish spread up the U.S. Atlantic coast and to Bermuda. In 2004, lionfish reached the Bahamas (Freshwater et al. 2009). Lionfish have now spread to the rest of the Caribbean islands (Schofield 2009), the coasts of Central America (USGS-NAS 2015), Florida Keys (Ruttenberg et al. 2012), and the Gulf of Mexico (Aguilar-Perera and Tuz-Sulub 2010). More recently, Ferriera et al. (2015) reported a confirmed lionfish capture in Brazil, south of the Amazon River outflow. Nucleotide and haplotype diversity for lionfish throughout the invasive range are moderate to low, suggesting that the now-extensive lionfish invasion originated from a very small founder population (Butterfield et al. 2015; Hamner 2007).

The proliferation of invasive species is typically aided by concepts within the enemy release hypothesis (ERH), which states that introduced species flourish in new surroundings due to the reduction of “enemies” (Keane and Crawley 2002). In the case of the invasive lionfish, this includes release from both predators and parasites. Lionfish are equipped with 18 venomous spines that deter predators (Morris 2009). The acetylcholine-based venom causes sufficient pain and localized swelling, but is non-lethal to humans if treated (Cohen and Olek 1989). Venomous spines, unfamiliarity to native predators, and aposematic coloration and form are the most probable reasons that lionfish have no known regular predators in their invaded range (Morris 2009). Grouper, sharks, and eels are considered the most likely candidates to become consistent predators of lionfish, but none of these species groups have been documented as regularly feeding upon them. Only on rare occurrences have there been reports of predation on uninjured lionfish; however, some groups believe that feeding native predators injured or dead lionfish will help them “acquire a taste” (Diller et al. 2014; Pimiento et al. 2012). In addition to this being dangerous for lionfish cullers, there is no indication that this method has been successful, yet most dive operators now condone the practice. Another competitive advantage that invasive species have over native reef fishes is the scarcity of parasites.
Invasive lionfish have fewer parasites in the western North Atlantic as compared to native fishes in the same range (Sikkel et al. 2014; Simmons 2014).

Invasive lionfish have rapidly spread throughout the tropical Western Atlantic because of their reproductive successes. They are gonochoristic batch spawners, in which the female releases two buoyant egg masses, which are externally fertilized by the male (Morris 2009; Morris et al. 2011). This reproductive strategy allows lionfish to reproduce throughout the year, as long as conditions are suitable. They are estimated to spawn every four days throughout the year, possibly assisting their rapid spread throughout the Caribbean region (Morris 2009). Studies examining lionfish fecundity show averages from about 24,000 to 75,000 eggs per female (Morris 2009; Priyadharsini et al. 2013), which is also dependent on individual size. Lionfish are also known mature very quickly: males can be sexually mature at just 100 mm TL, while females can be mature at 175 mm TL (Morris 2009).

Furthermore, invasive lionfish have been able to out-compete native fish for both food and space (Albins 2013, Albins and Hixon 2008; Green et al. 2012). In their invaded range, lionfish are known to exist in higher densities than in their native range (Darling et al. 2011). In Kenya and the Red Sea, for example, densities of 25 and 80 lionfish per hectare were reported, respectively (Darling et al. 2011; Fishelson 1997). In contrast, natural reef sites from North Carolina, Bahamas, and northern Gulf of Mexico have shown densities of 150, 393, and 49 lionfish per hectare, respectively (Morris and Whitfield 2009; Green and Côté 2009; Dahl and Patterson 2011). While densities were lower on natural Gulf of Mexico sites, they also reported densities of 1470 lionfish per hectare on their artificial sites (Dahl and Patterson 2011). According to recent studies in the Bahamas, lionfish have become 40% of the predatory fish biomass on their reef ecosystems; in turn, they have also reduced their prey fish biomass by an average of 65% (Green and Côté 2009; Green et al. 2012). Another study comparing predatory effects of lionfish versus the native coney *Cephalopholis fulva* on native prey fishes shows that the presence of lionfish negatively affects fish biomass, while the presence of coney did not have nearly the same affect (Albins 2013).

Lionfish are known to be voracious consumers in their invasive range, where their diet mainly consists of small reef fish and occasionally small invertebrates. They are
generalist predators, and consume about 40-70 different teleost species (Morris and Akins 2009; Muñoz et al. 2011). The primary teleost prey items depend largely on the local abundance because lionfish exhibit high site fidelity and generalist nature, and thus changes from area to area (Jasper 2013; Layman and Allgeier 2012; Morris and Akins 2009; Muñoz et al. 2011). The small-bodied species consumed by lionfish are also typical prey for the economically important competitors, such as large-bodied serranids and lutjanids (Morris and Akins 2009). This competition for food can create over-lapping niches and potentially decrease prey availability for native predators. Invasive lionfish are likely out-competing these native large-bodied predators (Albins 2013; Green et al. 2012). The effect of lionfish presence on economically important species is increasingly important from a management perspective, though lionfish rarely prey directly on these species (Morris and Akins 2009).

*Temperature Preference*

Cellular processes that occur within an organism are influenced by temperature (Evans and Claiborne 2006). Since lionfish are poikilothermic, their bodily processes are dependent on the surrounding water temperature. If temperatures change, the individual fish must either make adjustments internally (“acclimation”) or seek out more suitable conditions externally (“behavioral thermoregulation”; see Reynolds and Casterlin 1980).

Internally, the fish will involuntarily adjust biochemical processes at a cellular level to adapt to temperature changes via mechanisms such as homeoviscous adaptation (HVA) and homeophasic adaptation (HPA) (Evans and Claiborne 2006; Hazel 1995). These cellular-level responses are key to maintaining organismal equilibrium and functionality. Another means of responding to temperature changes is behavioral thermoregulation. Poikilothermic animals accomplish thermoregulation behaviorally by physically moving within an available temperature gradient to the most ideal temperature available (Reynolds and Casterlin 1979). In nature, individual fish utilize a combination of acclimation and behavioral thermoregulation to adjust to the changing environment.

Closely related to behavioral thermoregulation is the *temperature preference* of fish. Reynolds and Casterlin (1979) described temperature preference, “When presented a choice of ambient temperatures, as in a thermal gradient, motile organisms tend to
congregate in, or spend the most time in, a relatively narrow range of temperatures.”

There are two different concepts of temperature preference, *final preferendum* and *acute preferendum*. Final preferendum describes the temperatures at which the fish will aggregate, regardless of prior acclimation. This final preferendum temperature is thought to be species-specific and ideal to their physiological performance (Fry 1947; Reynolds and Casterlin 1979). Alternatively, acute preference is simply the range of temperatures at which fish congregate while under the influence of their prior acclimation temperature.

Behavioral thermoregulation has been measured in fishes with several methods. The oldest experimental designs used gradients to measure temperature preference. There are many variations of gradient tanks, from chambered devices to continuous vertical or horizontal gradients (McCauley 1977; Shelford and Allee 1913; Sullivan and Fisher 1953). In these experiments, individuals are presented with either chambers of different water temperatures or a gradient of water temperatures in a single tank. The fish are allowed to gravitate to their preferred temperature (McCauley 1977). Gradient methods often encountered problems with supersaturation of gases, maintenance of temperature gradients and other design flaws that influenced fish behavior (McCauley 1977). Another design is the use of shuttlebox methods. These designs have conditioned fish to perform a specific task to regulate their own temperature (McCauley 1977). Early designs required fish to press against a lever to control water temperature (Rozin and Mayer 1961). Neill (1972) introduced an apparatus called the “electronic shuttlebox”, which “allows fishes, by their spatial movement, to regulate the temperature in experimental tanks.” Since its first application, it has been used in a number of studies to measure preferences in temperature, salinity, and water turbidity (among other environmental variables; Meager and Utne-Palm 2008; Serrano and Serafy 2010).

Thermal preference is measured because it gives insight into the physiology and ecology of an organism. Regarding lionfish, acute preference measurements could correlate to field observations of distributions. Lionfish may be more likely to congregate in areas where water temperatures are within their *Zone of Final Preferendum* (Figure 1). This has been demonstrated in freshwater fishes and could be relevant to marine species as well (Cincotta and Stauffer 1984). Also, final preferendum has been linked to the temperature for optimal metabolic scope and optimal growth in fishes (Jobling 1981;
Metabolic scope, or the difference of oxygen uptake between a resting and active individual, can be maximized at a specific temperature (Kelsch and Neill 1990). In other words, an individual resting at this temperature is most efficiently utilizing their energy for metabolic demand, allowing for the surplus of energy to be applied to growth and reproduction. Jobling et al. (1981) supports this by showing that final preferendum in fishes is also correlated to the temperature for optimal growth in fishes, defined as the temperature at which the growth rate is highest under conditions of maximum feeding. In addition to metabolic scope, these temperatures are optimal for other biochemical and physiological processes in ectotherms, such as digestion, enzyme activity, or swimming speed (Hutchinson and Maness 1979; Kelsch and Neill 1990).

**Behavioral Tolerance**

The avoidance temperatures of an individual determine their behavioral tolerance range. Avoidance temperatures are measured because they quantify when a fish will likely move in the wild as a result of temperature. When temperatures become too warm or too cold for an individual, they instinctively seek out more favorable conditions or endure in their current location. Past conventional tagging studies have shown that lionfish demonstrate high site fidelity, but there is still evidence that lionfish may move substantial distances (Akins et al. 2014; Jud and Layman 2012). The reasons for lionfish movement in the wild are still unclear. Temperature may be a contributing factor to lionfish movement in certain habitats or regions. For example, lionfish avoidance temperatures would be increasingly relevant in areas of frequent and drastic temperature fluctuations, such as estuaries. Also, lionfish living near the edge of their thermal range would encounter extreme temperatures seasonally. Lionfish living in these regions, such as in North Carolina waters, may need to evade live threatening temperatures during winter months.
Figure 1. Diagram of thermal niche. Line “AP” is the regression line for the acute thermal preference, while line “LE” is the line of equality. The Zone of Final Preferendum is where these two lines intersect. Additional measurements in Jobling (1981) are critical thermal maximum as “CTM”, upper incipient lethal temperature as “UIT”, ultimate upper incipient lethal temperature as “UIDT”, and lower incipient lethal temperature as “LILT” (Figure from Jobling et al. 1981).
Physiological Tolerance

Fish not only have optimal temperatures for bodily processes, but also thermal limits at which those processes degrade or cease to function at all. Fish struggle at these extreme temperatures because of the effect temperature has on cellular proteins and enzymes. As temperature changes within a given range, proteins and enzymes needed for metabolism and normal cell function change shape or function. When temperatures near extremes, proteins and enzymes have difficulty performing their metabolic function, limiting the ability of the fish to survive. Heat shock proteins are generated within the cells in an attempt to maintain the regularity of cellular proteins and enzymes, so the individual fish may continue to function or survive until conditions return to a livable state (Roberts et al. 2010). At this point, the fish is exceeding its thermal limit and may not be able to maintain equilibrium or may experience muscle spasms (Lutterschmidt and Hutchinson 1997).

Critical thermal methodology (CTM) has been used for a number of decades as a method for measuring thermal tolerance in fishes. In a CTM experiment, temperature is increased or decreased from the acclimation temperature at a constant rate until a predetermined endpoint (Becker and Genoway 1979; Cox et al. 1974; Hutchinson 1961). This chosen endpoint is considered to be the physiological state where the fish would not be able to avoid death, should the temperature remain stable. At these extreme temperatures, their locomotory movements should be so disorganized that they would not be able to behaviorally thermoregulate or move to a more suitable environment (Cox et al. 1974). The most commonly used endpoints are loss of equilibrium (LOE) or the onset of muscle spasms (OS) (Beitinger et al. 2000; Lutterschmidt and Hutchinson 1997).

The one aspect of critical thermal methodology that receives the most scrutiny among fish physiologists would be the rate at which the temperature changes. The rates range from 1 °C per minute to 1 °C per hour, with the former being most common in the literature (Becker and Genoway 1979). Although there has been much debate over the proper rate, the ideal CTM rate would be one that is slow enough that the fish’s body temperature does not lag behind the water temperature, but is fast enough that the fish is not able to acclimate to the temperature changes (Beitinger et al. 2000). Becker and Genoway (1979) suggested the use of a rate of 0.3 °C per minute, based on a literature
review of past CTM studies and their own rate comparison study. They found that faster rates of temperature change, such as 1°C per minute, could result in heat shock or a lag of the fish’s body temperature behind the water temperature (Becker and Genoway 1979). In addition, it was difficult to accurately measure LOE and death at this rate. Becker and Genoway (1979) also demonstrated that rates of temperature change slower than 0.3°C per minute allowed fish to acclimate throughout the experiment. The 0.3 °C per minute rate has been frequently used for CTM experimentations in the subsequent literature, but other rates are still being employed as well. As examples, 0.3°C per minute was used in Currie et al. (1998) and Debnath et al. (2006), 1°C per minute was used in Rajaguru (2002), and 1°C per hour was used in Mora and Ospina (2001).

Critical thermal methodology offers insights into the ecological role of a species. Primarily, CTM describes the temperature at which fish of a certain species would not be able to survive. Therefore, when water reaches these high or low temperatures, it can be assumed that any fish exposed to this water would soon die or must move to more suitable temperatures (Hutchinson 1961). Temperature-related fish kills normally involve a closed environment, such as a river or lake, where the fish is unable to escape. However, in marine waters, fish kills related to temperature changes are almost exclusively caused by rapid onset cold-water events (Beitinger et al. 2000). This could be caused by either their ability to adjust to warmer temperatures more quickly or because cold water causes lethargic behavior, making it more difficult to move to warmer temperatures or increasing susceptibility to predation (Beitinger et al. 2000).

In addition to addressing ecological role questions for a single species, CTM is also frequently used as a tool for comparisons of thermal tolerance between species. Thermal tolerance is displayed using a thermal tolerance polygon, which encompasses the entire inhabitable thermal range of the species (Eme and Bennett 2009). It not only displays the CT<sub>min</sub> and CT<sub>max</sub> at different acclimation temperatures, but also quantifies the eurythermic potential of a species by calculating the area within the polygon (Eme and Bennett 2009). The dimensions of the thermal polygons are constructed by connecting the CL<sub>min</sub> and CL<sub>max</sub> of the species with the CT<sub>min</sub> and CT<sub>max</sub> regression (Eme and Bennett 2009).
However, the ecological relevance of CTM data has been debated since its introduction. Some critics note that conditions simulated by CTM experiments are rarely experienced by wild organisms and thus unproven (for example, see discussion in Lutterschmidt and Hutchinson 1997). This may be particularly true for organisms that generally are not physically “confined” to an area, such as marine fishes. However, for species that are able to live near their thermal extremes, there are indeed encounters with these life-threatening temperatures. The lionfish is a prime example of this type of species, as studies have shown that individuals in the invasive range live extremely close to their thermal limit (Kimball et al. 2004; Whitfield et al. 2014).

A trend among many tropical species (aquatic, marine, and terrestrial) is that they generally live closer to their thermal maximum and have a narrow thermal niche in comparison to temperate species (Tewksbury et al. 2008). Both of these characteristics stem from tropical climates being relatively stable in temperature year-round, or aseasonal (Madeira et al. 2012; Tewksbury et al. 2008). The species adapted to an aseasonal climate will be less suited for changing temperatures because their bodies are not internally prepared to adjust to them, compared to temperate species. Temperate species experience large changes of temperature every year, and thus have a wider thermal niche (Tewksbury et al. 2008). Aseasonal climates also allow tropical species to live closer to their thermal maximum, while rarely encountering temperatures above it. Thus, there is no need to be able to adapt to them (Tewksbury et al. 2008).

Few studies have examined the ecological effect of temperature on lionfish. In a study, conducted off North Carolina, researchers examined the transition of fish assemblages and lionfish abundance with depth (Whitfield et al. 2014). They found lionfish abundance increased with depth (most abundant at 38-46 m), as these depths maintained a winter mean above 15.3 °C and the shallower depths are too cold to overwinter. This study also suggested that increased ocean temperatures due to climate change might allow lionfish to overwinter in shallower water. The potential effects of climate change were also examined by Côté and Green (2012), who concluded that the lionfish larval duration would shorten, leading to an increase in the local retention of the individuals. Côté and Green (2012) also determined that lionfish predation rates on native fishes would increase in response to an increased metabolic rate.
In the western Atlantic, lionfish are currently established year-round from Cape Hatteras, NC through the southern Caribbean. North of Cape Hatteras, juvenile individuals are found seasonally in coastal habitats, which presumably die off during the winter months (Kimball et al. 2004). Since lionfish are a tropical/sub-tropical species, they probably only encounter temperatures approaching their thermal maximum in rare circumstances. Kimball et al. (2004) was the first to examine the effects of temperature tolerance on the invasive range of the lionfish. It investigated their chronic lethal minimum (CL\textsubscript{min}) to discuss the possible limits of their invasive range, reporting a CL\textsubscript{min} of 10.7 °C and cessation of feeding at 15.3 °C. Kimball et al. (2004) hypothesized that lionfish were limited by their inability to overwinter at temperatures colder than 12 °C. With this threshold, lionfish are able to overwinter as far North as Cape Hatteras, but only in deeper water. However, throughout Florida and the Gulf of Mexico, lionfish are able to overwinter at shallower depths. Kimball et al. (2004) also tested the effects of acclimation temperature and the rate of temperature change on their CL\textsubscript{min}, showing no difference between rates of temperature change (1 °C per day, 2 °C per day, and 3 °C per day) or acclimation temperatures (15 °C, 20 °C, and 25°C).

Understanding the relationships between physiology, behavior, and ecology provide additional insights into ecological problems, and provide the background for implementing the correct management techniques. This study aims to investigate the physiology and behavior of the invasive lionfish by describing their thermal niche. It defines three important biological parameters; temperature preference, behavioral tolerance, and physiological tolerance. Temperature preference data is used to determine final preferendum, which describes the temperature for optimal metabolic scope. Avoidance temperatures determine behavioral tolerance. These temperatures represent when an individual will move to seek out better conditions as a result of warming or cooling temperatures. CTM determines physiological tolerance, which are the temperatures that result in the death of the fish. The results serve as a platform for future research investigating the effect of temperature on this invasive species.

Acute preferences of lionfish were measured at different acclimation temperatures (20 °C, 25 °C and 32 °C). These acute preference measurements were used to graphically determine their final preferendum (Figure 1). In addition, differences in temperature
preference at different acclimation temperatures were tested. For poikilotherm fishes, acute preference is measured during the first two hours after being presented with a thermal gradient. During this time, their preference is under the influence of their acclimation temperature (Reynolds and Casterlin 1979). Therefore, we would expect to see differences in preference between lionfish acclimated to different temperatures. Avoidance temperatures were measured at three different acclimation temperatures (20 °C, 25 °C and 32 °C). Regression lines were developed from these measurements, and between these lines will be their behavioral tolerance range. Differences between avoidance temperatures at different acclimation temperatures were also tested. Lionfish were expected to display higher avoidance temperatures, as acclimation increases (Reynolds and Casterlin 1979). Expanding upon Kimball et al. (2004), CT_min and CT_max temperatures were recorded at different acclimation temperatures (13 °C, 20 °C, 25 °C and 32 °C) to establish the complete physiological tolerance range of the invasive lionfish. Differences in physiological tolerance in response to acclimation temperature were tested. According to Kimball et al. 2004, there were no significant differences in CL_min between different acclimation temperatures. The same is to be expected for CT_min and CT_max. To investigate how lionfish thermal tolerance compares to other tropical species, this study also measured the CT_min and CT_max for two other co-occurring tropical predatory fishes: schoolmaster snapper Lutjanus apodus and graysby grouper Cephalopholis cruentata. Eme and Bennett (2009) show similar temperature tolerance between reef-associated marine tropical fish species. Since the three species are primarily tropical, there should be no significant difference between their CT_min and CT_max.

Materials and Methods

Collection and Husbandry

Forty-two juvenile lionfish (87 mm-121 mm total length) were collected by SCUBA throughout southeast Florida and the Florida Keys. Hand nets were used for capture, and the individual lionfish are then transferred to a dry bag for the remainder of the dive. Fish were then taken back to the holding tanks at the Nova Southeastern University (NSU) Guy Harvey Oceanographic Center as soon as possible. About half
were collected by a team of Oceanographic Center graduate students and REEF (Reef Environmental Education Foundation) volunteers, diving underneath bridge pilings in the Florida Keys. Participants in REEF Lionfish Derbies located in Ft. Lauderdale, West Palm Beach, and Key Largo collected the other individual lionfish. A small number (n=4) were also purchased from “Carib Fish & More, Inc.” in Pompano Beach (these were caught by commercial collectors off the Broward County reef tract). A list of the individuals collected by location, size, and date is included as Table 1.

The fish were acclimated to four different temperatures before undergoing experimentation. Three large plastic water tanks were utilized for acclimation, each containing around 60 gallons of salt water, allowing for around 10 fish per tank at a time. Salinity was maintained between 32-36 ppt via occasional water changes and vigorous aeration ensured near saturation oxygen levels. UV sterilizers and biofiltration sustained optimal water quality. The acclimation temperatures were 13ºC, 20ºC, 25ºC, and 32ºC, chosen to represent typical water temperatures in New England, North Carolina, Florida, and the southern Caribbean region, respectively. Ideally, these temperatures were chosen for acclimating lionfish near the boundaries of their thermal range, but still are reasonably found in nature. The fish were acclimated to their respective temperatures 2-4 weeks before experimentation (Chung 1995; Segnini et al. 1993). They were fed ad libitum the same diet of feeder guppies *Poecilia reticulate*, goldfish *Carassius auratus auratus*, or frozen silversides from local pet stores over the course of their holding and acclimation.

**Electronic Shuttlebox: Preference and Behavioral Tolerance**

The experimental shuttlebox tank (Figures 2 and 3) included two circular chambers with a passage between them, allowing the fish to move freely between the two chambers. Infrared (IR) lights shined from directly underneath the tank, which was detected by an IR-sensitive camera above the tank. The fish creates a “shadow,” blocking the infrared light and allowing the computer to monitor the position of the fish via the contrast difference between dark shadow and the well-lit background of the tank. Temperature fluctuation was controlled by a computerized system, which pumps cold or warm water into the tank, depending on the position of the fish.
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Table 1. List of 42 lionfish identification numbers, collection site, date of collection, and acclimation temperature. Fish size is measured after completion of the experiments or death.
Figure 2. A diagram of the electronic shuttlebox (Horodysky 2013). The experimental “dumbbell-shaped tank” contains the specimen. The fish can move freely between the “hot” or “cold” side of the experimental tank. The mixing towers allow for the dynamic changes in temperature on each side of the experimental tank.
Figure 3. Photo of the shuttlebox as assembled at the NSU Oceanographic Center, *circa* 2013. Image includes the “dumbbell-shaped tank” and the mixing towers behind. Not pictured are the infrared lights underneath the tank and the sensors above, the chiller/heater systems, or the computer and control switches for the mixing tower flows.
Lionfish were individually removed from their acclimation tanks for experimentation. Each lionfish underwent two 12-hour phases of experimentation: the first was the acclimation phase, then followed secondly by the dynamic phase. The acclimation portion occurred over night and maintained two invariant temperatures, separated by a temperature difference of Δ3 ºC between the two tanks. One side of the tank was designated the “hot” side and the other is the “cold” side. During the acclimation stage, the temperature of each side of the tank was set around the acclimation temperature of the fish. Lionfish acclimated to 20 ºC were set to 18.5 and 21.5 ºC (Δ3 ºC), and those acclimated at 25 ºC were set to 23.5 and 26.5 ºC. Meanwhile, due to how close the acclimation temperatures were to their thermal limits, lionfish acclimated to 13 ºC had tank temperatures set to 13 to 16 ºC (Δ3 ºC), and those acclimated to 32 ºC were set to 29 to 32 ºC. This phase allowed the fish to sense and learn that there was a “hot” and “cold” side of the tank, and that it could move freely between them. After 12 hours, the acclimation phase ended, and the dynamic portion began.

The dynamic phase involved temperature changes in both sides of the tank in response to the movement of the fish. When the fish was on the “hot” side of the tank, temperature in each chamber continually increases, while still maintaining Δ3 ºC. While the fish is on the “cold” side of the tank, temperatures continually decreased in the same fashion. System-wide minimum and maximum temperatures were set at 10.7 ºC and 36.7 ºC, respectively. The lionfish undergo this experimentation for 12 hours or until the fish lost equilibrium, although only the first two hours of dynamic data were used for the acute preference measurement. Following experimentation in the shuttlebox, each lionfish was tagged using small, plastic T-bar tags (Hallprint, Ltd.; Australia), allowing for identification of individual fish for the remainder of experimentation.

.
For measurement of acute preferences, only the first two hours of dynamic data were used from each lionfish shuttlebox experiment. According to Reynolds and Casterlin (1979), preference measurements taken during this time frame are strongly influenced by prior acclimation temperatures. Temperature was recorded roughly every second based on where the lionfish was located within the experimental tank. Avoidance temperatures were determined as the temperature when the subject switched sides. These temperatures were taken during the same two-hour dynamic phase.

Analysis of the acute preference data was conducted by measuring the mean of all the temperature values that each lionfish occupied during the experiment to get a single value. A nonlinear second polynomial (quadratic) line was fit to these data. Using the graphical process employed by both Reynolds and Casterlin (1979) and Jobling (1981), the zone of final preferendum was determined as the point along the acute preference line that intersects with the line of equality (LE; Figure 4). The line of equality represents the point where acclimation temperature is equal to the acute preferendum (Cincotta and Stauffer 1984; Jobling 1981). Linear regression lines were also fit for the upper and lower avoidance temperatures. These data were tested using one-way ANOVAs to determine whether lionfish preference and avoidance were significantly different at each acclimation temperatures.

**Critical Thermal Methodology (CTM): Physiological Tolerance**

Juvenile lionfish were collected in the same manner as previously mentioned. Schoolmaster and graysby were captured live via hook and line from Broward county reefs and estuaries. Air bladders of captured lionfish were vented, if necessary, to ensure their survival. Lionfish were acclimated to four different temperatures (13°C, 20°C, 25°C, and 32°C) before undergoing the experiment, while the other species were only acclimated to 25°C. The fish were acclimated to their respective temperatures 2-4 weeks before experimentation, allowing for proper time to acclimate to their respective temperatures (Chung 1995; Segnini et al. 1993). They were fed *ad libitum* a diet of live feeder guppies, live goldfish, or frozen silversides *Menidia menidia* from local pet stores over the course of their holding and acclimation.
Figure 4. The Zone of Final Preferendum was determined by constructing the diagram above. It shows the relationship between acclimation temperature and preference temperature for white sucker *Catostomus commersonii*. The curved line is the quadratic model used to fit the plotted points and the straight line is the line of equality. They intersect at 27.10 °C. (Figure from Cincotta and Stauffer 1984).
Each fish was randomly selected for testing in either the acute CT_{min} or CT_{max} trials. Half of the fish in each acclimation temperature underwent the CT_{min} trial, while the other half underwent the CT_{max} trial. Each trial occurred in thermostatically controlled plastic containers holding clean seawater. Water was continuously aerated and circulated to maintain homogenous oxygen and temperature levels throughout the container. Temperature began at the fish’s acclimation temperature and increased or decreased by 0.3 °C per minute, as suggested by Beitinger et al. (2000). Since the acute critical thermal method (CTM) was being employed, the endpoint of the experiment was non-lethal. The endpoint was defined as when the fish achieved a loss of equilibrium (hereafter, “LOE”; i.e., it cannot maintain dorso-ventral orientation for 1 minute, per Beitinger et al. 2000).

Upper and lower thermal tolerance for each replicate treatment group were calculated as the mean of the CT_{min} or CT_{max} trials per Eme and Bennett (2009); similarly, the grand mean of the collective replicate endpoints are the CT_{min} or CT_{max} for the temperature acclimation regime (Cox 1974). Thermal tolerance polygons were constructed for lionfish by connecting the CT_{min} and CT_{max} with CTM regressions to produce a quadrilateral figure (Figure 5). Similarly, these polygons demonstrated an intrinsic tolerance zone (i.e., thermal tolerance independent of previous thermal acclimation) and acquired tolerance zones (i.e., thermal tolerance gained through acclimation) by dividing polygons with horizontal lines from extrapolated CT_{min} and CT_{max} values at CTM limits. An additional thermal tolerance polygon was constructed (per the methods of Eme and Bennett [2009]) for the purpose of comparing polygonal area (°C^2). This additional polygon was formed by connecting CL_{min} (from Kimball [2004]) and CL_{max} (estimated to be 35 °C) with the CTM regressions.

Data analysis was conducted using R software (version 3.1.2; R Development Core Team 2014). Two separate ANOVAs were utilized to detect differences in mean CT_{min} and CT_{max} values between lionfish acclimated to different temperatures. Normality of residuals was tested using the Shapiro-Wilk test and homogeneity of variances was tested using Bartlett’s test. ANOVAs were conducted to detect differences in mean CT_{min} and CT_{max} values between the different fish species.
Figure 5. Example of a thermal polygon (figure from Eme and Bennett 2009). The top and bottom lines represent the CT$_{\text{max}}$ and CT$_{\text{min}}$ regression lines for the common goby *Bathygobius fuscus*. Black circles represent actual CTM measurements. White circles represent the predicted CTM measurements at their highest and lowest theoretical acclimation temperature, determined by the CL$_{\text{min}}$ and CL$_{\text{max}}$. 
Results

The second order polynomial model of the lionfish acute preference data is displayed by the following equation: $y = -0.0729x^2 + 3.9981x - 26.007$. The acute preference quadratic line and the line of equality intersect at 28.7 °C, estimating 27.7-29.7 °C (or 28.7 ± 1 °C) to be the zone of final preferendum for lionfish (Figure 6). Statistical analysis with the ANOVA found there to be no significant difference in temperature preference with acclimation temperature (p>0.05; Table 2).

Linear regressions for upper and lower avoidance temperatures of lionfish were fit to the data (Figure 7). Upper avoidance data yielded an R-square value of 0.11 (y=0.1745x + 25.21), while lower avoidance showed an R-square value of 0.14 (y=0.1938x + 18.42). The ANOVAs for both upper and lower avoidance showed significant differences between avoidance temperatures at different acclimation temperatures (p<0.05; Table 3 and Table 4).

Lionfish displayed an absolute $C_{T_{\text{max}}}$ of 40 °C and an absolute $C_{T_{\text{min}}}$ of 9.5 °C. $C_{T_{\text{max}}}$ and $C_{T_{\text{min}}}$ were different for lionfish acclimated to different temperatures (ANOVA, p<0.05; Table 5 and Table 6). $C_{T_{\text{max}}}$ for lionfish ranged from 40 °C to 30.5 °C and the linear regression had an R-square value of 0.97 ($y = 0.501x + 23.975$). $C_{T_{\text{min}}}$ ranged from 16.5°C to 9.5 °C and the linear regression displayed an R-square value of 0.92 ($y = 0.4273x + 2.0228$). The thermal polygon showed a total estimated polygonal area of 573 °C² (Figure 8). Comparisons of thermal limits between species show a difference in $C_{T_{\text{max}}}$ at 25 °C (ANOVA, p<0.05; Table 7), but no difference in $C_{T_{\text{min}}}$ (ANOVA, p>0.05; Table 8). The schoolmaster $C_{T_{\text{max}}}$ (39.9 ± 0.67 °C) was 1.4 °C higher than graysby (38.5 ± 0.53 °C) and 2.8 °C higher than lionfish (37.1 ± 0.25 °C). However, the lionfish $C_{T_{\text{min}}}$ (12.7 ± 0.52 °C) is 0.58 °C lower than graysby (13.3 ± 0.46 °C) and 0.40 °C lower than schoolmaster (13.1 ± 0.42 °C) (Figure 9). The entire inhabitable range at this acclimation temperature for lionfish, graysby, and schoolmaster is 24.5 °C, 25.3 °C, and 26.9 °C respectively.
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Table 2. ANOVA summary for the effect of acclimation temperature on temperature preference.

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Table 3. ANOVA summary for the effect of acclimation temperature on upper avoidance.

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Table 4. ANOVA summary for the effect of acclimation temperature on lower avoidance.

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Table 5. ANOVA summary for the effect of acclimation temperature on CT_{max}.

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Table 6. ANOVA summary for the effect of acclimation temperature on CT_{min}.  

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Table 7. ANOVA summary for the effect of species on CT$_{\text{max}}$.

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Table 8. ANOVA summary for the effect of species on CT$_{\text{min}}$. 
Figure 6. Thermal preference graph for lionfish, including the Zone of Final Preferendum (ZFP) at the intersection of the acute thermal preference line (AP) and line of equality (LE). Zone of Final Preferendum was determined to be 28.7 ± 1 °C.
Figure 7. Behavioral tolerance range of lionfish, featuring the upper avoidance regression line (UA) and the lower avoidance line (LA). Triangles represent upper avoidance data points, while circles represent lower avoidance data points.
Figure 8. Invasive lionfish thermal polygon, showing the $CT_{\text{max}}$ and $CT_{\text{min}}$ regression lines. Total estimated polygonal area is $573 \, ^\circ \text{C}^2$. 
Figure 9. CT_{max} and CT_{min} comparisons of lionfish compared to two other species. All subjects were acclimated to 25 °C. Significant differences were found between the CT_{max} values of the three species. CT_{min} values were not significantly different between species.
Discussion

Temperature Preference

The zone of final preferendum for lionfish defined by this study was estimated to be 27.7-29.7 °C (81.9-85.5 °F), which generally describes either a warm sub-tropical summer or a tropical winter in the western north Atlantic. Other tropical marine species have displayed similar temperature preferences. Measurements of juvenile pebbled butterflyfish (Chaetodon multicinctus), banded damselfish, (Abudefduf abdominalis) and convict tang (Acanthurus triostegus sandvicensis) are 27.0 °C, 30.2 °C, and 29.3 °C, respectively (Medvick and Miller 1979). Some tropical marine fish have shown a lower final preferendum, such as the juvenile humpback grouper (Epinephelus altivelis) and adult yellow tang (Zebrasoma flavescens), which exhibited 24.5 °C and 21 °C, respectively (Reynolds and Casterlin 1980). These studies utilized similar shuttlebox procedures, but measured final preferendum directly instead of determining final preferendum from acute preferences.

Final preferendum correlates well with optimal temperature for metabolic scope, growth, cardiac performance, swimming activity, and digestion (Brett 1971; Jobling et al. 1981; Kelsch and Neill 1990). A previous study also investigated the bioenergetics of lionfish, relating temperature to their feeding rate (Cerino et al. 2013). This study determined that the 29.1 °C was the optimal temperature of feeding for lionfish, which is within our estimated final preferendum. It appears that the invasive lionfish are at their physiological optimum when living near this temperature range.

Acute preference data showed significant amounts of variation. Acclimation temperature explained about 15% of the lionfish’s movements in the shuttlebox, while 85% of the variation remains unexplained. In comparison, Cincotta and Stauffer (1984) reported R-squared values from 0.40 to 0.78, meaning acclimation temperature accounted for 40%-78% of their fish movement. However, the Cincotta and Stauffer (1984) results were from gradient preference measurements, instead of shuttlebox measurements. Shuttlebox studies have reported differing amounts variation in shuttlebox behavior. For example, Mortensen et al. (2007) reports an R-squared value of 0.48 when analyzing the relationship between temperature preference and specific growth rate. Other studies
report large amounts of temperature preference variation between individuals, mentioning individual behavior and exploratory movement as possible explanations (Killen 2014; Konecki et al. 1995). Measuring fish behavior is extremely difficult because their actions are often unpredictable, even when controlling for as many variables as possible. For statistical testing, there was no significant difference of acute preference between acclimation temperatures. The test results were likely due to a low sample size and high variation between the points, since a power test revealed only 27% probability of detecting a significant difference.

The electronic shuttlebox measured thermal preferences and avoidance temperatures for 20 lionfish. A total of 34 fish were tested in the shuttlebox, with 20 of those successfully demonstrating behavioral thermoregulation (59%). There are a couple of possible explanations for the 14 lionfish that did not thermoregulate. First, lionfish have the tendency to demonstrate high site-fidelity, meaning they prefer to stay still rather than explore (Jud and Layman 2012). Reduced exploratory movement could hinder their ability to learn the shuttlebox system, as they would have less of an opportunity to discover the difference in temperatures between the tanks. Second, due to the design of the shuttlebox, small water current is created within each side of the tank. In currents, lionfish tend to lie close to the substrate and avoid movement (James Morris, NOAA, pers. comm. 2014). This small current could discourage lionfish from exploring the tank.

Some of lionfish spent long lengths of time at very high temperatures due to the rate of change slowing within the shuttlebox chambers. When the shuttlebox reached temperatures nearing 37 ºC, the rate of temperature change slowed down, due to the fact that the “hot bath” was only a few degrees higher than the actual temperature. A few subjects could withstand these temperatures and would not move to the other tank, despite showing the capability of doing so earlier in the experiment.

**Behavioral Tolerance**

Mean upper avoidance temperatures ranged from 28.1-31.0 ºC, while mean lower avoidance temperatures ranged from 22.1-24.5 ºC. Significant differences existed between avoidance temperatures at different acclimation temperatures, demonstrating that lionfish behavior is affected by surrounding water temperatures. The regression lines
for upper and lower avoidance were used to demonstrate the lionfish behavioral tolerance range (Figure 10). Using the regression lines as a predictor, the maximum and minimum avoidance temperatures are 32.2 °C and 20.5 °C, respectively. This range is much narrower, in comparison to the physiological tolerance range of the invasive lionfish. In total polygonal area, behavioral tolerance is actually just 26% of the total physiological tolerance. This means that lionfish, when given the opportunity, will stay well away from their thermal limits. When inside of this behavioral tolerance zone, it is likely that lionfish are physiologically unaffected by the surrounding water temperature. Outside of the behavioral tolerance zone is the resistance zone, utilized by Jobling (1981; Figure 11). At these temperatures lionfish can survive, but their physiological performance may be limited, with potential deleterious effects for fitness (Kimball et al. 2004).

**Physiological Tolerance**

Lionfish in the present experimental trials displayed differences in $CT_{\text{min}}$ and $CT_{\text{max}}$ in response to changing acclimation temperatures. This is contrary to the $CL_{\text{min}}$ results reported by Kimball et al. (2004). One possible reason for this could be the rate of temperature change. This study used a rate of 0.3 °C per minute, while Kimball et al. (2004) used 1 °C per day (0.0167 °C per minute). The slower rate used in the present study presumably allows the lionfish to acclimate to the changing temperatures throughout the experiment, masking any effect of prior acclimation. Kimball et al. (2004) also reports a mean $CL_{\text{min}}$ of 10.7 °C, in comparison to a mean $CT_{\text{min}}$ of 12.67 °C in this study (prior acclimation at 25 °C). The difference in these results is most likely due to a combination of slower temperature rate of change and the endpoint. Kimball et al. (2004) used death as the endpoint, while this study used LOE. This is not surprising, as LOE always comes before death, allowing for time to pass (and temperature to decrease) in between. Although Kimball et al. (2004) did not state specific temperatures of LOE; they did report that temporary and permanent LOE occurred just prior to death. It is likely that their LOE temperatures would be lower than the $CT_{\text{min}}$ reported by the current study.
Figure 10. Behavioral tolerance range (shaded area) shown in relation to physiological tolerance (dashed lines). Upper avoidance (UA) and lower avoidance (LA) regression lines form the upper and lower boundary of the behavioral tolerance range.
Figure 11. Tolerance zone comparison, showing the resistance zone (shaded gray region), behavioral tolerance zone (striped region), and the zone of final preferendum (black region).
Lionfish responses were consistent with other CTM studies of tropical fishes. Eme and Bennett (2009) portrayed thermal tolerance polygons of five tropical mangrove-associated and reef-associated fish species. Similar to the lionfish, these five species also displayed differences in CT$_{\text{min}}$ and CT$_{\text{max}}$ with acclimation temperature. Additionally, Eme and Bennett reported overall polygon area (reported as °C$^2$) as a comparative measure for eurythermy between species. The mangrove-associated species showed higher polygonal area than the reef-associated species. The increased eurythermy demonstrated by these mangrove species is likely due to their exposure to frequently changing temperatures from tidal movement.

Using the CL$_{\text{min}}$ from Kimball et al. (2004) and an estimated CL$_{\text{max}}$, overall polygon area was determined for lionfish (Table 9). A polygon area of 573 °C$^2$ for lionfish is smaller than the tropical mangrove-associated species (~820 °C$^2$), but higher than the reef-associated species (~430 °C$^2$) (Table 9). This result suggests that lionfish are not necessarily more eurythermal than all tropical species, but may be slightly more eurythermal than Atlantic reef-associated species.

When discussing these CTM data and their potential ecological significance on the boundaries of lionfish, it may be most effective to use the constructed thermal polygon as a predictor. Using the results of this study in this way can improve our understanding of the invasive lionfish’s thermal limits and the potential effect of global climate change on their range.

The current overwintering limit in the northern hemisphere is Cape Hatteras, North Carolina (Kimball et al. 2004), and this point typically serves as the northern-most boundary for other reef-fishes as well. The average water temperature during 2014 off Beaufort, NC was 17.6 °C (NOAA National Oceanographic Data Center). Using the CT$_{\text{min}}$ regression line and 17.6 °C as the acclimated temperature, 9.5 °C is the predicted CT$_{\text{min}}$ for lionfish in this region. Even with ocean temperatures expected to increase by at least 2-3 °C in the next 50-100 years (2.7°C according to IPCC in 2014, Pörtner et al. 2014), it will still not be warm enough north of this boundary to allow lionfish to overwinter absent a biochemical change within the species to facilitate low-temperature survival (Hoegh-Guldberg et al. 2007; Rummer et al. 2014). According to NOAA NODC data, Virginia and New Jersey have average coastal winter water temperatures (January-
<table>
<thead>
<tr>
<th>Species</th>
<th>Primary Habitat</th>
<th>Total Polygonal Area ($\text{C}^2$)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common goby <em>Bathygobius fuscus</em></td>
<td>mangrove/tidepool</td>
<td>828</td>
<td>Eme and Bennett (2009)</td>
</tr>
<tr>
<td>Square-tail mullet <em>Liza viagiensis</em></td>
<td>mangrove/tidepool</td>
<td>823</td>
<td>Eme and Bennett (2009)</td>
</tr>
<tr>
<td>Sandflat goby <em>Bathygobius sp.</em></td>
<td>mangrove/tidepool</td>
<td>639</td>
<td>Eme and Bennett (2009)</td>
</tr>
<tr>
<td><strong>Lionfish</strong> <em>Pterois spp.</em></td>
<td>reef</td>
<td>573</td>
<td><strong>Present study</strong></td>
</tr>
<tr>
<td>White-tailed humbug <em>Dascylus aruanus</em></td>
<td>reef/seagrass</td>
<td>442</td>
<td>Eme and Bennett (2009)</td>
</tr>
<tr>
<td>Nine-banded cardinalfish <em>Ostorhinchus novemfasciatus</em></td>
<td>reef/seagrass</td>
<td>408</td>
<td>Eme and Bennett (2009)</td>
</tr>
</tbody>
</table>

Table 9. Comparisons of total polygonal area ($\text{C}^2$) between lionfish and other tropical species measured by Eme and Bennett (2009). The mangrove and tidepool-associated species show higher total polygonal area than reef-associated species. The total polygonal area of lionfish is higher than reef-associated species, but lower than mangrove/tidepool-associated species.
March) of 4.6 and 3.7 °C, respectively. This shows that these water temperatures would have to increase by at least 5 °C for lionfish to overwinter in these areas, which is higher than most predicted water temperature changes (Hoegh-Guldberg et al. 2007; Pörtner et al. 2014). However, increasing ocean temperatures could allow lionfish abundance to rise closer to the North Carolina shore, especially during the colder winter months (Whitfield et al. 2014).

The southern boundary of the invasive lionfish range as of 2014 was off the coast of South America, east through Venezuela to Trinidad and Tobago (USGS-NAS 2015). Some have questioned whether lionfish will expand their invasive range down the East coast of Brazil due both the Amazon-Orinco Plume and the North Brazil Current (Luiz et al. 2013). However, the first lionfish was recorded off the coast of Brazil at Arraial do Cabo in May of 2014 (Ferreira et al. 2015). According to the measured CT_{min}, lionfish would likely expand their overwintering range to the coast of Uruguay, similar to the predictions of Morris and Whitfield (2009) and Miloslavich et al. (2011).

The threat of increased ocean temperatures from global climate change has prompted studies and discussions of the upper thermal tolerance of reef-fish and other reef-inhabiting organisms (Booth et al. 2011; Figueira and Booth 2010; Hoegh-Guldberg et al. 2007; Mora and Ospina 2001; Ospina and Mora 2004; Rummer et al. 2014). There is some disagreement as to whether reef-associated fishes will be affected by an estimated increase in ocean temperatures of 2-3 °C over the next 100 years (Mora and Ospina 2001; Rummer et al. 2014). When examining CT_{max} data, it appears unlikely that the abundance of many species will be significantly affected by a temperature change of 2-3 °C. Most species have CT_{max} values about 8 °C above the average Caribbean sea surface temperature (SST) (Mora and Ospina 2001). In the case of the invasive lionfish, they will likely be unaffected by increasing ocean temperatures at this latitude, since their CT_{max} in this area is around 36-40 °C. However, indirect effects of increasing temperatures could still influence the populations of reef-associated fishes in these areas (including lionfish) through such problems as habitat degradation, coral bleaching, and ocean acidification (Hoegh-Guldberg et al. 2007).
This study also examined the differences in CTM at 25 °C between three different reef-associated species, lionfish, graysby, and schoolmaster. Lionfish displayed the smallest tolerance range (degrees from CT_{min} to CT_{max}) of the three species at 24.5 °C, while the others were only larger by 1-2 °C. This is likely because lionfish do not have larger thermal tolerance ranges, but actually very similar ranges compared to other tropical reef species (Eme and Bennett 2009). Based on the described ranges within the literature, all three tested species can each live year-round from North Carolina to South America; therefore the similarity of tolerance range should not be surprising (Allen 1985; Kimball 2004; Rocha et al. 2008).

Past studies have also used CTM on tropical fishes to examine thermal tolerance. Mora and Ospina (2001) tested the CT_{max} of 15 difference reef fishes with a rate of 1 °C per hour at an acclimation of 26.5 ± 0.5 °C. This study described CT_{max} that ranged from 34.7°C to 40.8°C, with estuarine and intertidal species having higher CT_{max} values. Another study investigated thermal maxima in tropical estuarine fishes, showing CT_{max} between 39.5-44.5 °C (Rajaguru 2002). Although it is unclear why CT_{max} varies from species to species, it may be because of their primary habitat. Estuarine species are subjected to more fluctuating temperatures while reef species inhabit a more stable environment. The fluctuating temperatures of an estuary system could explain the difference in thermal limits, either because these fish are more accustomed to changing temperatures or they actually experience a wider range.

*Thermal Niche*

This study defines the entire thermal niche of the invasive lionfish (Figure 12). This allows for a better understanding of the invasive lionfish and their interaction with temperature. Figure 12 describes how a lionfish would be affected by temperature under certain circumstances. For example, an invasive lionfish in warm tropical temperatures (30 °C) would have a temperature preference around 27.5 °C, CT_{min} around 14.75 °C, and CT_{max} around 39 °C. Figure 12 also shows the relationship between the different
Figure 12. Lionfish thermal niche incorporates the linear regression lines of CT$_{\text{max}}$, CT$_{\text{min}}$, upper avoidance (UA) and lower avoidance (LA). The nonlinear quadratic line for acute thermal preference (AP) is also included. Also shown is the line of equality (LE) and Zone of Final Preferendum (ZFP), which intersect at 28.7 °C.
measurements collected during this study. It appears that lionfish prefer temperatures that are closer to their thermal maximum than their thermal minimum.

Additionally, this study demonstrates the differences between measuring physiological parameters versus behavioral parameters. The difference of slopes between the thermal limits (physiological) and preference/avoidance temperatures (behavioral) indicate that lionfish thermal limits were more affected by the acclimation temperature. Also, the thermal limits measured showed much less variation than the preferences and avoidances. Behavioral studies traditionally have high amounts of variation because of the unpredictability of a live animal. There are many unknown factors that contribute to the actions of an individual and it is very difficult to understand the driving factors behind their behavior.

**Conclusion**

For the invasive lionfish, temperature can define geographic range, impact physiological processes, and influence behavior within their range. This study further elucidates the relationships between the invasive lionfish and temperature. The temperatures at which lionfish are at their physiological optimum, their behaviorally selected tolerance range, and their physiological limits are now established for juveniles. Investigating the underlying physiology and behavior behind the lionfish is important to understanding the possible ecological effects of this invasion. Our thermal information will be key to predicting and analyzing future geographic range changes for the invasive lionfish and investigating their behavioral response to temperatures within their habitats.
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