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Ecophysiology of lionfish metabolic and visual systems: Are there physiological limits to inshore invasion?

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

Ecophysiology of lionfish metabolic and visual systems: Are there physiological limits to inshore invasion?

By

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Abstract

Lionfish (*Pterois* spp.), an invasive species native to the Indo-Pacific, have permanently established themselves throughout the greater Caribbean, Gulf of Mexico, and regions of the Western Atlantic ranging from as far north as North Carolina to central Brazil. As their fundamental range expands, lionfish threaten to migrate into estuarine environments as they have been found to tolerate low salinities and an eclectic range of temperatures. The physiological capacity of invasion was assessed by quantifying the visual ecology of lionfish utilizing corneal electroretinography (ERG) as well as their metabolic scope and hypoxia tolerances under various temperature-oxygen-regimes utilizing intermittent-flow respirometry. Seasonal changes in temperature-dissolved oxygen levels consistent with Atlantic/Gulf of Mexico inshore estuaries not only exceed the physiological tolerances of lionfish, but also constrain metabolic scope at sub-lethal levels by significantly limiting maximum metabolic rate across all temperatures. Median S_{crit} values were 33%, 39%, 46%, and 54% at 15, 20, 25, and 30℃ respectively. Luminous sensitivities, temporal resolutions (Flicker fusion frequency), and spectral sensitivities scaled similarly with other estuarine piscivores indicating lionfish possess a visual system that can function effectively within estuarine photic conditions. Overall, visual characteristics of estuaries will not pose as a significant barrier to lionfish, but minimum winter temperatures and hypoxia will pose controlling and limiting factors substantially preventing further inshore invasion. However, caution should still be advised as lionfish may capitalize on specific temporal and spatial scales that provide suitable habitat quality and abundance of prey items. Further insight is needed to forecast the effects of temperature-dissolved oxygen on lionfish metabolic-scope.

Keywords: Lionfish, invasion, estuaries, ecophysiology, vision, metabolic-scope

Acknowledgements

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Discussion

Introduction

Lionfish (*Pterois* spp.) are invasive fish species originally from the Indo-Pacific and are now well established throughout the western Atlantic Ocean from Cape Hatteras, North Carolina to central South America (González et al. 2009; Whitfield et al. 2002; Ferreira et al. 2015). Lionfish are broadly considered a significant threat to native fish populations and local biodiversity because of their rapidly expanding geographic range and growing populations, high predation and fecundity rates, lack of natural predators, and trophic cascading impacts, including the reduction of coral cover on coral reefs (Fishelson 1997; Green et al. 2012; Morris 2012; Albins and Hixon 2013). These ecological attributes and life history characteristics contribute to their success and high capacity for invasion, which is considered to be one of the top 15 emerging global environmental issues (Sutherland et al. 2010).

Lionfish commonly associate with tropical and sub-tropical coral reefs but have been found in other habitats including mangrove systems, seagrass meadows, estuaries, and artificial structures (Barbour et al. 2010; Jud et al. 2011). Individuals have been shown to tolerate salinities as low as 4‰ (~10% seawater) (Jud et al. 2014) and populations have been established *ca.* 4 km inshore in the Loxahatchee River estuary, Florida (Jud et al. 2011). Lionfish therefore appear to be physiologically equipped to invade U.S. South Atlantic Bight (from Florida through North Carolina) and Gulf of Mexico estuaries. Although 10˚C is known to be the lethal minimum temperature for lionfish, Barker et al. (2014) has shown that this threshold can be surpassed with gradual acclimation. Thus, low winter temperatures may be ineffective at preventing permanent establishment since a number of inshore estuaries remain above this minimum threshold (i.e., Cape Fear estuary (NC), Pamlico Sound estuary (NC), Charleston Harbor estuary (SC)) (Dame et al. 2000; Kimball et al. 2004). Temperature is thought to be one of the only abiotic factors controlling lionfish distributions on a large scale, and with ongoing increase in ocean temperatures, lionfish can be expected to distribute further inshore and northward (Whitfield et al. 2014). Lionfish can reduce native fish recruitment on coral reefs by up to 79% (Albins and Hixon 2008), leading to concerns about their impacts on estuarine ecosystems. Estuaries are important nursery and habitat areas for many commercially, recreationally, and ecologically important native fish species (Beck et al.

2001; Courrat et al. 2009). It is therefore critical to understand and predict the ability of lionfish to occupy inshore estuarine areas.

Previous physiological work by Cerino et al. (2013) investigated lionfish respiration rates to construct a bioenergetics model and found that populations of lionfish at reported densities in the Caribbean are estimated to remove approximately half a ton of prey fish per hectare of reef habitat per year. Additionally, Cerino et al. (2013) found that respiration and consumption rates are highly temperature dependent. Barker et al. (2014) furthered this investigation by depicting the thermal niche of lionfish using thermal tolerance polygons determining that these fish display an acquired thermal tolerance since physiological and behavioral tolerances increase with coinciding acclimation temperatures. We continued this line of investigation by using stop-flow respirometry to characterize the effects of temperature lionfish energetics and hypoxia tolerance, and standard electroretinography (ERG) to characterize visual capabilities. Our overall object was to clarify if lionfish could be effective colonists in Atlantic and Gulf of Mexico inshore estuaries, as well as to predict the types of environments lionfish can and cannot successfully invade.

The standard metabolic rate (SMR) of fish is the rate of oxygen consumption at rest in a post-absorptive state; whereas the maximum metabolic rate (MMR) is the maximum rate of oxygen consumption a fish can achieve. An organism's metabolic scope is defined as the difference between maximum and standard metabolic rates and therefore confines the total amount of energy that can be apportioned to growth, reproduction, and movement (Fry 1947). Metabolic scope of lionfish can be applied to the description of life history, ecology, and behavior in energetic terms to determine the effects from environmental variation (Kerr and Warner 1980; Horodysky et al. 2015).

Multiple biotic and abiotic factors influence the metabolic scope of fishes, including temperature, salinity, pH, dissolved oxygen, pollutants, diseases, parasites, and light levels (Jonassen et al. 2000; Wuenschel et al. 2004; Fitzgibbon et al. 2007; Horodysky et al. 2015). The interaction of temperature and dissolved oxygen has received considerable attention in particular due to its spatial and temporal variability in aquatic ecosystems (Taylor and Peck 2004). This variability is especially true of estuarine

environments given the large range of natural temperature fluctuations. Episodic hypoxia (lasting minutes to days) is also a naturally occurring phenomenon in estuaries (Breitburg 1992; Rabalais et al. 2010), particularly along the U.S. Atlantic coast during the warmer months of summer and early autumn, when the decomposition of organic matter consumes dissolved oxygen and the density-driven stratification of the water column isolates bottom water from exchange with the oxygen-rich surface water (Bishop et al. 2006; Tyler et al. 2009). Temperature is a controlling factor that drives biochemical reactions and sets standard and active metabolic rates whereas dissolved oxygen is a limiting factor that interferes with the ability of the cardio-respiratory system to deliver oxygen to the tissues, thereby reducing active metabolic rate and constraining metabolic scope (Fry 1947; Horodysky et al. 2015). This research aims to elucidate the controlling and limiting effects of temperature and dissolved oxygen levels on the aerobic-metabolic scope of lionfish to provide mechanistic form-function insights on the fundamental niches, habitat use, and capacity for invasion into estuarine ecosystems.

Stop-flow respirometry was used to examine the effects of temperature and hypoxia on the aerobic metabolic scope of lionfish with the objective of determining the ability of these fish to function under temperature-oxygen conditions common in western Atlantic estuaries. Nothing is currently known about the aerobic metabolic scope of lionfish or the effects of temperature and hypoxia on their aerobic scope. We hypothesized that lionfish will be very intolerant of hypoxia because they evolved in the well-oxygenated and fairly shallow waters of the Indo-Pacific; the founder populations of lionfish in the western Atlantic also came from these same environmental conditions. More specifically, we hypothesized that episodic hypoxia, much like the challenging visual conditions in estuaries, will create a mismatch between environmental conditions and lionfish physiological capabilities that will limit their spread into these ecologically critical areas.

Virtually all behavioral decisions are based on information transmitted through sensory systems (Browman 2005; Dangles et al. 2009). While significant information exists on visual function in many temperate and tropical reef species, visual function in lionfish is completely unstudied. Maintaining optimal visual performance in inshore

estuarine waters is a difficult task since these habitats are some of the most variable, photodynamic, aquatic habitats on earth (Horodysky et al. 2010). This variability encompasses vertical mixing, stratification, wave activity, clouds and weather, sunrise and sunset, seasonal solar irradiance, phytoplankton dynamics, as well as anthropogenically induced processes such as eutrophication and sedimentation (McFarland and Loew 1983; Wing et al. 1993; Schubert et al. 2001; Gallegos et al. 2005; Kemp et al. 2005; Horodysky et al. 2010). Because visual systems of fishes generally reflect the characteristics of aquatic light fields they inhabit (Guthrie and Muntz 1993), we hypothesized lionfish are likely visual predators and will be challenged in turbid estuarine environments as their visual systems evolved for the warm, bright, clear waters of their native tropical Indo-Pacific reefs. To test this hypothesis, we characterized their visual systems by measuring three visual parameters using standard electroretinography (ERG): response to light intensity, speed of vision, and wavelength sensitivity in juvenile and adult lionfish collected in Florida. Achromatic and chromatic sensitivities as well as temporal resolutions are fundamental predictors of teleost light niches and feeding ecologies. The former predictors will be invaluable metrics to describe the functions and tasks of lionfish visual systems, allowing for comparison to various known estuarine species (Horodysky et al. 2008; 2010; 2013).

Materials and Methods

All husbandry and experimental protocols were approved by the Nova Southeastern University (protocol number DK2) and the College of William and Mary (protocol number 12610) Institutional Animal Care and Use Committees and followed all relevant laws of the United States.

Acquisition of Lionfish

Approximately 30 lionfish (80-250-mm total length, 38g-140g) were acquired with hand nets off the coast of the Florida Keys and maintained at the Nova Southeastern University (NSU) Guy Harvey Oceanographic Center in Dania Beach, Florida. Fish were maintained in recirculating aquaria at 25±2°C and 20±2‰ to resemble estuarine conditions. Animals were kept under natural photoperiods from indirect ambient sunlight transmitted through the windows of the holding facility. Diets of experimental subjects

comprised a mixture of live grass shrimp (*Palaemonetes paludosus*), frozen bay scallops, shrimp, and sardines fed *ad libitum* three times weekly. Fish were acclimated for a minimum of two weeks before use in experimental trials. After the initial acclimation period, a subset if fifteen subjects were transported in temperature controlled aerated coolers to the Virginia Institute of Marine Science (Gloucester Point, Virginia) for experiments on visual function.

Electroretinography

Electroretinography (ERG) was used to examine visual function. The electroretinogram represents the summed potentials of various cell types within the retina (Brown 1968; Ali and Muntz 1975) and is considered a useful tool for addressing questions about visual function (Ali and Muntz 1975; Horodysky et al. 2008). ERG experiments were conducted on ten fish utilizing the procedures described Horodysky et al. (2008; 2010; 2013). Subjects were removed from the holding tanks during daylight hours, sedated with an intramuscular injection of Ketastet (ketamine hydrochloride, 30mg kg^{-1}), and immobilized with an IM injection of the neuromuscular blocking drug Flaxedil (gallamine triethiodide, 10mg kg^{-1}). Recording of vertebrate neural waveforms in anaesthetized and immobile subjects is a common practice to minimize the obscuring effect of muscular noise or overt movements (Hall 1992; Parkyn and Hawryshyn 2000; Horodysky et al. 2008). At the conclusion of all experiments, fish were immediately euthanized with a massive overdose (350 mg kg^{-1}, or .10x the anesthetic dose) of sodium pentobarbital.

After being anesthetized, fish were transferred to a light-tight experimental enclosure and placed on a cloth sling within a rectangular Plexiglas tank so that only a minimal portion of the head and the eye remained above the surface. Subjects were ventilated with temperature-controlled (25±2˚C), aerated seawater to minimize any confounding effects of temperature on ERG recordings (Saszik and Bilotta 1999; Fritsches et al. 2005) and subsequently dark adapted for a minimum of 60 minutes. Experiments were conducted during daylight hours since lionfish are known crepuscular hunters (Green et al. 2011). The assessment of visual function in lionfish consisted of three types of experiments that assess different functions of their visual system: the range

of light sensitivity, the speed of vision, and the range of wavelength (color) sensitivity. These visual properties are fundamental predictors of teleost light niches and feeding ecologies (Horodysky et al. 2008; 2010; 2013). Teflon-coated, silver-silver chloride wire electrodes were used to measure corneal potentials by placing the active electrode on the corneal surface and implanting the reference electrode in the incurrent nostril. The system was grounded with a 6cm x 26cm stainless steel plate. ERG signals were amplified with a DAM50 amplifier (World Precision Instruments, Sarasota, FL, USA) using a 10,000x gain, with a 1Hz high pass and 1kHz low pass filter. Amplified ERG signals were further filtered with a HumBug® active electronic filter (Quest Scientific, N. Vancouver, BC, Canada) to remove periodic electrical noise, and were digitized at 1kHz sampling frequency with a 6024E multifunction DAQ card (National Instruments, Austin, TX, USA). ERG recordings and stimulus presentations were controlled using software written in LabVIEW (National Instruments, Austin, TX, USA).

Luminous Sensitivity

To assess luminous sensitivity, lionfish were presented with incremental increases of white light stimuli to generate an intensity-response curve, referred to as a "V log I curve" (Horodysky 2008; Kalinoski et al., 2014). "V" refers to the amplitude (in :volts) of the "b" wave of the ERG response, and "I" light intensity (Evans et al. 1993). A custom-built single LED light source (working range ~1 to 1×10^4 cd m^{-2}), with an attached diffuser and collimating lens to produce an even illumination field, was used to generate the stimulus (Kalinoski et al., 2014). The light source was positioned so that the entire eye was exposed to light stimuli. The analog output of the DAQ card controlled the absolute brightness of the lamp and combinations of 1.0 and 2.0 log unit neutral density filters (Kodak Optical Products, Rochester, NY, USA) were used to further adjust the range of light intensities, as described in Kalinoski et al., (2014). Light intensities were calibrated using an International Light (Peabody, MA, USA) IL1700 radiometer.

Experiments progressed from subthreshold to saturation intensity levels in 0.2-log unit steps. At each intensity step, ERG b-waves were recorded from a train of five 200-ms flashes, each separated by 200-ms rest periods. The b-wave amplitude in response to the last light flash was used for subsequent analysis. This process was repeated five times

and the responses were averaged. The data were subsequently normalized to the maximum voltage response (V_{max}), such that V_{max} = 100%. Interspecific comparisons of relative luminous sensitivity were made at stimulus irradiances eliciting 50% of V_{max} (K_{50}; Horodysky et al. 2008). Dynamic ranges were calculated as the log irradiance range between the limits of 5-95% V_{max} (Frank 2003; Horodysky et al. 2008). This assay characterizes the range of sensitivity and may be used to identify minimum thresholds of visual discrimination as well as maximum intensity thresholds above which retinal sensitivity will be at least temporarily diminished (Horodysky et al. 2008; Brill et al. 2008).

Temporal Resolution

To measure temporal resolution, flicker fusion frequency (FFF) experiments employed the LED system used in the intensity-response experiments in conjunction with methods described by Fritsches et al. (2005). This assay quantifies the ability of a visual system to track white light flickering in logarithmically increasing frequencies across a range of light intensities. Sinusoidally-modulated white light stimuli, ranging in frequency from 1 Hz (0-log units) to 100 Hz (2.0-log units), were presented to subjects in 0.2-log unit frequency steps, repeated five times at each frequency, and responses averaged. Light stimuli trains were presented for 5 s, followed by 5 s of darkness. For each subject, six total FFF experiments were conducted: one at 25% and 50% of maximum stimulus intensity (I_{max}) from the V logI curve, and one in each of log10 step intervals up to four orders of magnitude of light intensity. To determine a subject's FFF threshold at a given intensity level, the power spectrum of the average responses (signal) were analyzed and the power of the predominant frequency compared to the power of a neighboring range of frequencies (noise). FFF was taken as the frequency at which the power of the response signal fell below the power of the noise. The FFF threshold at I_{max} is the maximum FFF attainable by a visual system. The FFF threshold at I_{25} is assumed to be a proxy for a visual system's FFF threshold at ambient environmental light intensity (Horodysky et al. 2008) and was used as a metric for interspecific comparisons.

Spectral Sensitivity

Spectral sensitivity was assessed using methods described in Horodysky et al (2008; 2010). This assay characterizes the range of wavelengths discriminated, and in concert with luminous sensitivity, these data can be used to calculate contrast discrimination thresholds and predator-prey visual interaction distances in waters of different color and turbidity to make interspecific comparisons of relative sensitivities (Nilsson et al. 2014). The output of a Cermax Xenon fiberoptic light source (ILC Technology, Sunnydale, CA, USA) was controlled by a CM110 monochromator, collimated, and passed through each of two AB301 filter wheels containing quartz neutral density filters (CVI Laser Spectral Products, Albuquerque, NM, USA). The first wheel allowed light attenuation from 0 to 4 log units in 1 log unit steps and the second in 0 to 1 log units of light intensity in 0.2 log unit steps. In concert, the two wheels allowed the attenuation of light from 0 to 5 log units in 0.2 log unit steps. Stimuli were controlled by a LabVIEW program controlling a Uniblitz LS6 electronic shutter (Vincent Associates, Rochester, NY, USA). A cylindrical lens focused the attenuated light beam onto the entrance slit of the monochromator to produce mononchromatic light. A 1 cm diameter quartz light guide was placed within 10 mm of the subject's eye. Approximately isoquantal light stimuli covering the spectral range from UV (300 nm) to the near infrared (700 nm) were presented to subjects sequentially in 10 nm steps *via* the selective use of neutral density filters. Subjects were presented with five single 40-ms stimulus flashes at each wavelength, each followed by 5 s interval of darkness. ERG b-wave response amplitudes were recorded and averaged to form raw spectral response curves for each fish. Spectral V log I curves were performed at the wavelength of V_{max} and response voltages were transformed to sensitivities by converting the former to equivalent intensities through the equation:

$$S = 100x10^{|I_{max} - I_N|} \quad (1)$$

Where S is sensitivity, I_{max} is intensity at maximum response voltage, and I_N is the intensity at response voltage (Horodysky et al. 2008). Final sensitivity curves were expressed as percentages (100% = maximum sensitivity). Spectral sensitivity curves for all subjects were then averaged and normalized to the maximum response value.

Spectophotometry of Ocular Media

To assess the wavelengths passed by the lionfish ocular media, the cornea, vitreous humor, and lens were dissected from three euthanized subjects not used in ERG trials. Dissected components were immediately transferred to UV transmitting cuvettes within a 0.9% saline solution and placed in a Shimadzu BioSpec-1601 spectrophotometer so that the measuring beam passed through the tissue. Absorbance and transmission were recorded from 300 – 700 nm.

Respirometry

Four different temperature regimes (15˚C, 20˚C, 25˚C, and 30˚C) were used to depict the thermal tolerance range of lionfish and temperature fluctuations consistent of Atlantic and Gulf of Mexico inshore estuaries (Kimball et al. 2004). Eight individuals were subjected to three separate trials to determine aerobic scope under normoxic and hypoxic conditions, critical oxygen saturation level (S_{crit}) at each temperature.

Metabolic rate

We determined the aerobic metabolic rate of lionfish under the various temperature-hypoxia regimes using an intermittent-flow respirometry system (Schurmann & Steffensen 1997, Horodysky et al. 2011, Roche et al. 2013). The fish were acclimated for a minimum of two weeks at the respective temperature and fasted for a 48-hr period before use in an experiment. The intermittent-flow respirometry system was as described Lapointe et al. (2014) encompassing a cylindrical respirometry chamber (4.6 l volume, Loligo Systems) constructed of plexiglass, accompanied with a fiber optic oxygen sensor and respective oxygen meter (PyroScience, Aachen, Germany) to measure oxygen levels. The oxygen sensor was mounted in an air-tight, T-joint hose-barb fitting inserted in the water recirculation tubing and the digital output from the meter was recorded with computers running AquaResp2 (DTU-Aqua, www.aquaresp.com/). Oxygen saturation (%) values were converted to oxygen content (mg O_2 l^{-1}) using standard equations (Steffensen 1989) within the AquaResp2 software routines. The respirometer was submerged in an outer water bath where it could be flushed intermittently with temperature-controlled, filtered, aerated seawater. The water in the outer bath was

bubbled with nitrogen to control oxygen levels during hypoxic trials. Nitrogen flow was regulated by solenoid valves which were controlled by AquaResp2 software. During recording and flushing cycles, the water within the respirometer was thoroughly mixed using an external recirculating pump (Steffensen 1989). The respirometer was covered with black plastic to minimize visual disturbances and the external water bath was covered with a plastic sheet to reduce gas exchange at the air-water interface. The outer bath water temperature was modulated by an external chiller and 300-W electric aquarium heater and an electronic controller.

Metabolic rate (MO_2) measurements were made on approximate ten-minute cycles consisting of a five-minute flush period and two-minute equilibration interval, followed by a specifically designated measurement interval to allow oxygen levels within the respirometer to be reduced by approximately 10%. After the data recording interval, the AquaResp2 software executed a linear regression of the oxygen levels against measurement time. AquaResp2 determined MO_2 from the following equation:

$$MO_2 = \frac{V_{rem} x\ \beta\ x\ \Delta PO_2}{m_f\ x\ \Delta t} = \alpha\ x\ \frac{V_{rem\ x\ \beta}}{m_f} \quad (2)$$

Where V_{rem} is the respirometer volume corrected for fish volume (calculated from fish mass and assuming 1 g of fish mass = 1 ml), β is the oxygen solubility in water, m_f is the weight of the fish in kg, t is time, and α is the slope calculated by the linear regression equation. Background oxygen consumption ($\Delta[O_2]t^{-1}$) was measured as the decline in oxygen levels within the respirometer when a fish was not present and was accounted for by subtracting it from the $\Delta[O2]t^{-1}$ when a fish was in the respirometer.

Standard metabolic rate in normoxia

Standard metabolic rate in normoxia (SMR_N) was determined by calculating the average of the 10 lowest MO_2 values recorded during the last 24 hours of the 48-h period for each individual fish that was in the respirometer (Schurmann & Steffensen 1997; Claireaux et al. 2000; Lapointe et al. 2006; 2014).

Critical Oxygen Saturation

Critical oxygen saturation (S_{crit}) values were determined by decreasing the oxygen level in the respirometer in a step-wise fashion until the aerobic metabolic rate declined in unison with further reduction in ambient oxygen under each temperature regime (Schurmann & Steffensen 1997). Up to four MO_2 measurements were taken at each of nine different levels of oxygen saturation (90, 80, 70, 60, 50, 40, 30, 20, and 10%) which included oxygen levels at which the fish could no longer maintain SMR. Individual S_{crit} values were calculated using a two-piece segmented regression (SegReg, available at: https://www.waterlog.info/segreg.htm) (Fig. 1). S_{crit} trials took approximately 8-12 h per fish and were followed by full oxygen saturation of the respirometer and the fish left undisturbed until the next day, when the fish were subsequently transferred to the chase tank and allowed to recover for 24 h.

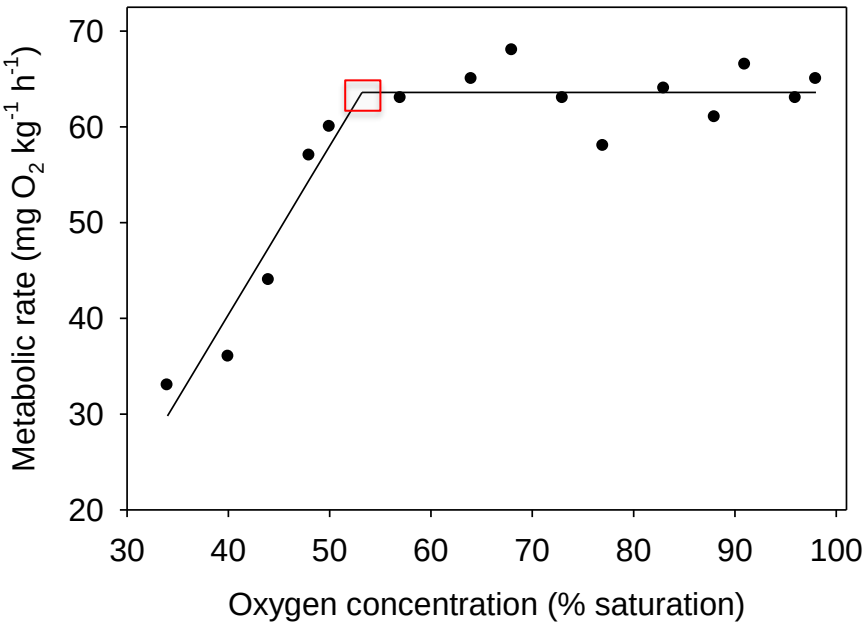


Figure 1 Example of a piece-wise segmented regression of an individual lionfish after an S_{crit} experiment at 25°C. The black lines represent the separate regressions of stable SMR (horizontal line) and when SMR can no longer be maintained (diagonal line). The red square indicates the critical oxygen saturation value of the individual which is a common metric of hypoxia tolerance in fishes. Metabolic rate measurements are in mg O_2 kg^{-1} h^{-1}.

Maximum Metabolic Rate in Normoxia

Individual fish were introduced into a large chase tank set at the respective acclimation temperature where they were gently prodded to induce burst swimming until the point of exhaustion. This was marked by no apparent response when the fish were handled or removed from the water (Black 1958; Lapointe et al. 2006; 2014). Fish were weighed and immediately transferred to the respirometer where MO_2 was measured continuously for the next 48 hours or until little to no variation in response occurred. Maximum metabolic rates (MMR_N) were designated as the highest MO_2 measured over the first 12 hours of the experiment following methods described in Lapointe et al. (2014).

Maximum Metabolic Rate under Mild and Severe Hypoxia

After the fish had been allowed to recover from the procedures used to measure MMR in normoxia, they were allocated a minimum one-week rest period. Individuals were subjected to the same chase protocol described above and then exposed to a predetermined level of hypoxia which was 15-20% O_2 saturation greater than the subject's S_{crit}. MO_2 measurements were taken from the time of transfer until the MO_2 stabilized for a maximum of 12 h. MMR_H under mild hypoxia was depicted as the highest MO_2 recorded during that 12-h period following similar methods by described by Lapointe et al. (2014).

Calculated Parameters and Data Analysis

Aerobic scope was calculated by subtracting an individual's respective SMR from MMR and relative metabolic scope was calculated as the ratio of MMR to SMR. To evaluate the effect of temperature, dissolved oxygen level, the temperature/dissolved oxygen interaction on metabolic scope, S_{crit}, and relative metabolic scope, one-way repeated measures ANOVAs were conducted to elucidate any significant differences in SigmaPlot 11.0 (Systat Software). All tests used the 5% significance level and the normality and homogeneity of variance criteria were verified using the Shapiro-Wilk and Levene Median tests.

Results

Electroretinography

Luminous Sensitivity

Induced white-light ERG b-wave responses increased with stimulus intensity to median maximum amplitudes (V_{max}) at 1.6 ±0.1 log units (Fig. 2) where they proceeded to decline due to photoreceptor hypersaturation. K_{50} values, frequently used for interspecific comparisons, normalized from the *V/logI* curve produced a median of 0.24 ± 0.05 log units and a median dynamic range, defined as 5 – 95% of V_{max}, of 1.98 log units (-0.77 to 1.20 ± 0.05) (Fig 3). Lionfish K_{50} values were found to be significantly different to all of the fishes examined in Horodysky et al., (2008; 2010) with the exception of summer flounder (*P. dentatus*) (Fig. 3). Both of these species elicited relatively smaller responses at 50% of I_{max} and were therefore left-shifted compared to the majority of other estuarine fishes.

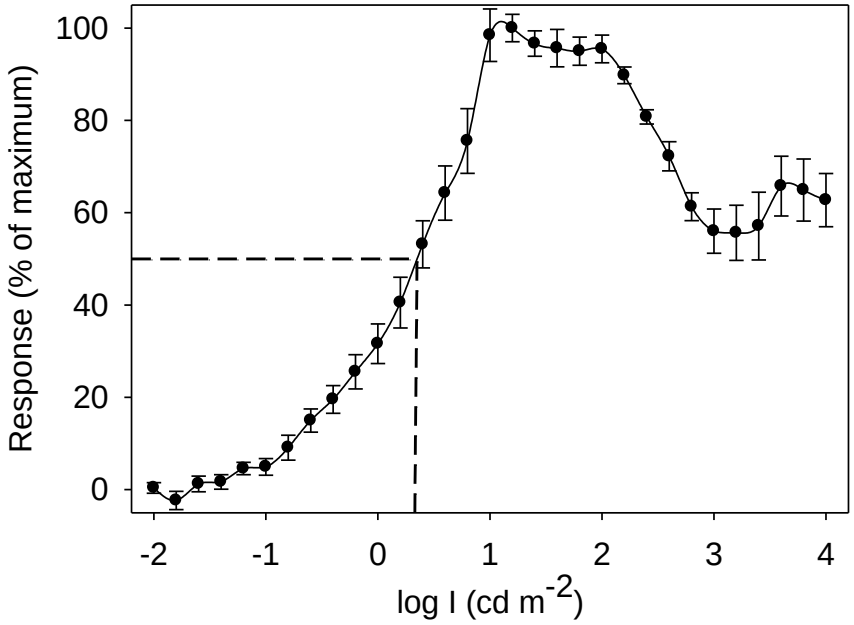


Figure 2 Intensity-response *V/LogI* curve averaged from nine individual lionfish. Responses were normalized to the maximum response voltage for each individual. The dashed line represents the lionfish K_{50} value (illumination at 50% V_{max}). Error bars are representative of ± 1 s.e.m. Light intensities are in log candela m^{-2}

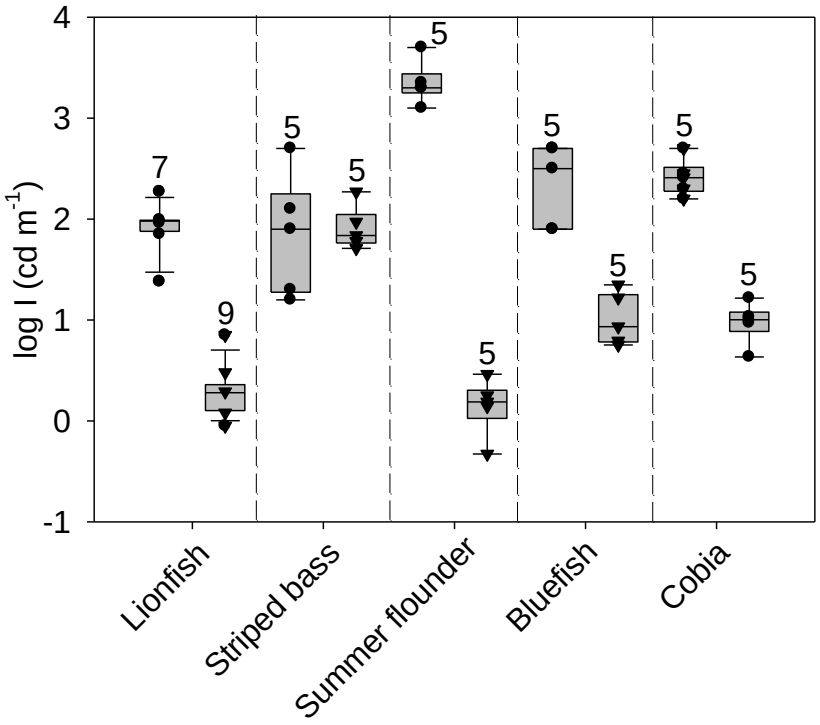


Figure 3 Interspecific comparisons of individual lionfish K_{50} values (represented by filled in triangles) and dynamic ranges (circles) versus those of various estuarine fishes previously published in Horodysky et al., (2008; 2010); consent to use these data was granted by the Journal of Experimental Biology. Median values are represented by the horizontal center line within each box. Numerical values above the error bars signify (n) individuals tested for the respective species. The grey rectangular boxes span the first quartile to the third quartile with the whiskers showing the minimum and maximum values excluding outliers. Outliers are data points above or below the whiskers for each box.

Flicker Fusion Frequency

Median FFF of individual lionfish were observed at 25% and 50% of their respective normalized V_{max} to quantify visual temporal resolution under estuarine-proxy light levels. Median FFF responses observed at 25% were 35 ± 4 Hz, and at 50% Vmax; 40 ± 3 (Fig. 4). FFF values at ambient estuarine light intensities (I_{25}) were not

significantly different compared to the four estuarine piscivores (bluefish, striped bass, cobia, summer flounder) in Horodysky et al., (2008; 2010) (Fig. 4). Lionfish median FFF values at 100% of V_{max} (85.5 ±7.5 Hz), however, were significantly different from bluefish, cobia, and summer flounder, but not that of striped bass (76 ±2.71 Hz) (Fig. 4).

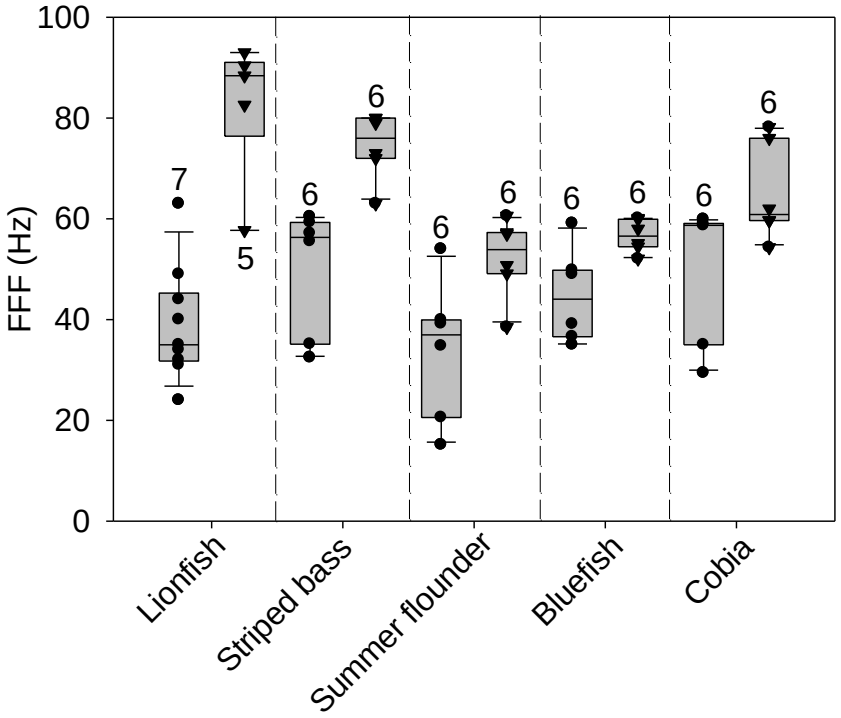


Figure 4 FFF values at 25% of V_{max} (data indicated by filled circles) representing ambient environmental light levels (I_{25}) and 100% of V_{max} (data indicated by filled triangles) representing an environment where light is not limited of lionfish, versus those of various estuarine fishes [previously published in Horodysky et al., (2008; 2010); consent to use these data was granted by the Journal of Experimental Biology]. . Numerical values above the error bars signify (n) individuals tested for the respective species.

Spectral Sensitivity

Table 1 SSH and GFRKD rhodopsin template estimations fitted to ERG data based on Akaike's information criterion (AIC) for maximum likelihood. AIC values weighted the GFRKD trichromatic condition the highest of all conditions tested (-186) and predicted a short-wave alpha-pigment in the UV spectrum (381 nm), an intermediate wavelength pigment (434 nm), and a longwave pigment (512 nm). The intermediate and long wavelengths are associated with blue and green colors respectively

Condition	Template	$8_{max,1}$	$8_{max,1}$	$8_{max,1}$	-log(*L*)	P	AIC	)AIC
Mono	GFRD	505			-48	2	-90	96
	SSH	504			-55	2	-104	82
Di, ∀	GFRD	411	510		-91	5	-172	14
	SSH	410	510		-91	5	-173	13
Di, ∃, S	GFRD	460	513		-96	6	-181	5
	SSH	411	510		-91	6	-170	16
Di, ∃, L	GFRD	461	513		-96	6	-181	5
	SSH	439	513		-79	5	-147	39
Di, ∃, B	GFRD	498	520		-95	6	-177	9
	SSH	437	512		-72	6	-130	55
Tri, ∀	GFRD	384	443	513	-98	7	-183	3
	SSH	**381**	**434**	**512**	**-100**	**7**	**-186**	**0**

Spectral sensitivity responses of lionfish spanned from 350 ± 2 nm to 630 ± 1 nm with peak median sensitivity at 490 ± 5 nm (Table 1, Fig. 5). The spectral sensitivity curve was optimally fitted using an GFRKD template based on Akaike's information criterion (AIC) maximum likelihood value (-1.86) to estimate specific pigment mechanisms within the lionfish eye (Table 1). The template predicted trichromacy with an intermediate wave α-band pigment, (λ_{max}= 434 ± 2.6 nm), a longer-wave α-band pigment (λ_{max}= 512 ± 1 nm), and a short wave α-band pigment (λ_{max}= 381 ± 2 nm) (Table 1, Fig. 5).

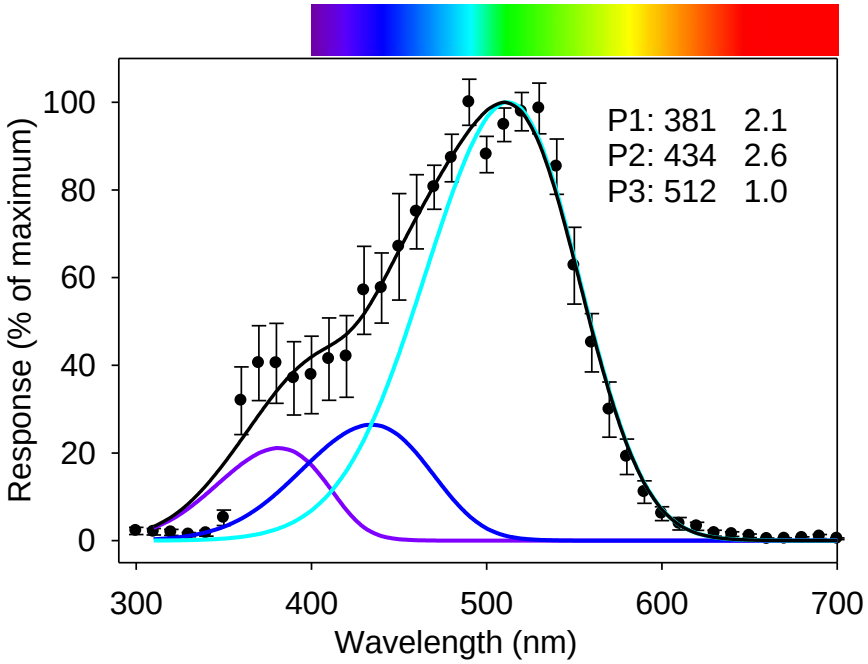


Figure 5 Spectral sensitivity curves of seven individual lionfish calculated from the electroretinograms (ERGs).. Responses at each wavelength were averaged and normalized to the maximum voltage response of each individual. Error bars represent ±1 s.e.m. SSH pigment estimation templates were fitted by maximum-likelihood based on lionfish ERG data. Values to the right of each pigment label are estimated λ_{max} and pigment weight is estimated by the model where P1 is a β-band of the longest wavelength pigment (P3 - Green)), and P2 (Blue) is the intermediate wavelength pigment.

Spectrophotometry of Ocular Media

Spectrophotometric examination of lionfish lens and cornea displayed maximum transmittance under the predicted blue-green photopigments (440-500 nm) (Fig. 6). Significant transmittance at UV wavelengths is in agreement with the presence of a short-wave length pigment (P1) with a maximum absorbance at 381 nm (Figure 6).

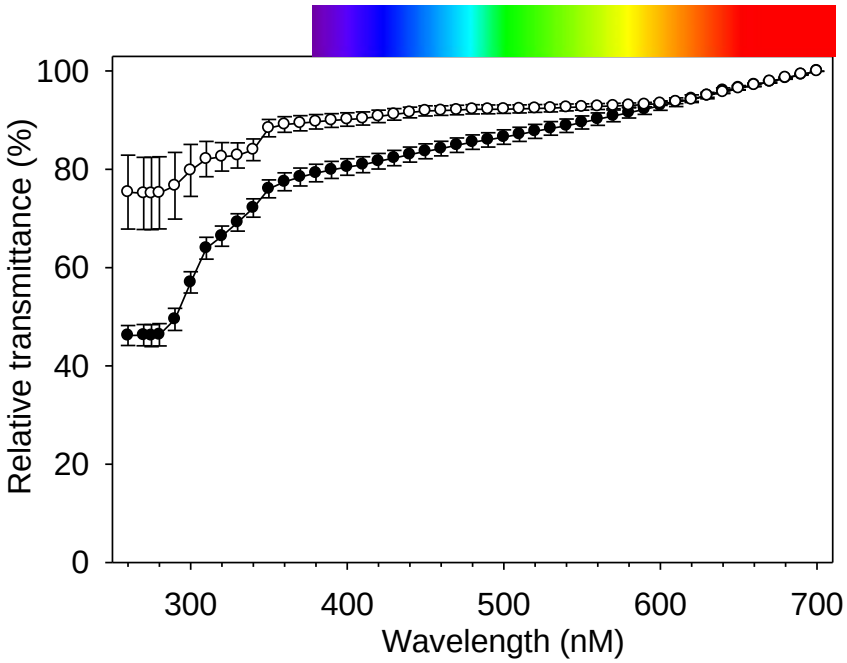


Figure 6 Spectrophotometric results illustrating the transmittance of lionfish ocular media under varying wavelengths. Maximum transmittance can be seen under blue-green wavelengths with heavy absorption occurring at shorter wavelengths

Effects of oxygen levels on SMR, MMR, and metabolic scope

Standard metabolic rates were only significantly different at increased temperatures, 25℃ and 30℃, which were also significantly different from each other (Fig 7). Hypoxia did not have a significant effect on SMR with the exception of the 15℃ temperature trials where there appeared to be an increase in SMR likely resulting from high sensitivity to hypoxia eliciting avoidance behaviors. Lionfish median SMRs were 27.1 ±3.5, 31.2 ±3.1, 65.5 ±6.6, and 131.1 ±6.7 mg O_2 kg^{-1} h^{-1} at 15, 20, 25, and 30℃ respectively (Fig 7). Median Q_{10} values (the factor which metabolic scope changes per 10℃ change in temperature; Prosser, 1973) between 15 - 25℃ and 20 - 30℃ were 2.4 and 3.6 respectively, indicating that increases in temperature towards the optimal end of the lionfish temperature range produce higher SMRs with a ten-degree temperature difference compared to the rate of increase at lower temperatures.

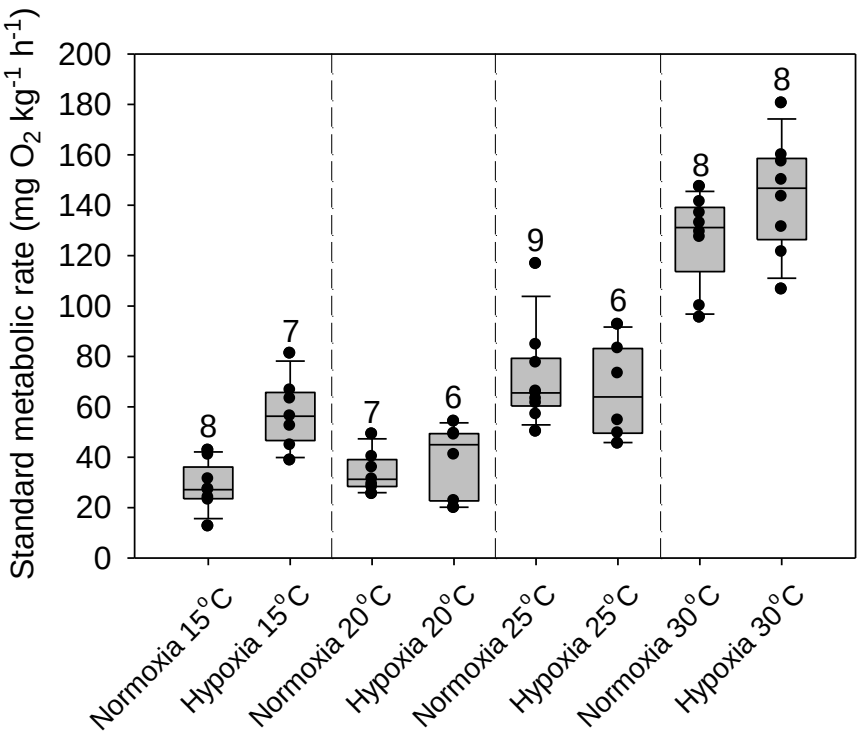


Figure 7 Standard metabolic rate displayed of individual lionfish over various temperature regimes comparing SMRs under normoxic conditions vs. hypoxic conditions. Median values are represented by the horizontal center line within each box. Numerical values above the error bars signify (n) individuals tested for the respective species. The grey rectangular boxes span the first quartile to the third quartile with the whiskers showing the minimum and maximum values excluding outliers. Outliers are data points above or below the whiskers for each box. Units are in mg O_2 kg^{-1} h^{-1}

Maximum metabolic rates (MMR) were found to be significantly different across all temperatures with the exception of 15℃ and 20℃ (Fig 8). Lionfish MMR increased rapidly at higher temperatures as evidenced by the Q_{10} values (2.1 and 3.4) between 15-25℃ and 20-30℃ respectively. Mild hypoxia significantly depressed MMR across the intermediate temperatures (20 and 25℃) but not for the lowest and highest temperature regimes. Under normoxic conditions, median MMRs were 88.0 ±8.8, 111.4 ±8.2, 176.9 ±7.5, 238.8 ±13.4 mg O_2 kg^{-2} h^{-1} at 15, 20, 25, and 30℃ respectively while under hypoxic

conditions MMRs were 77.3 ±4.6, 69.7 ±1.2, 95 ±6.9, 211.5 ±9.0 mg O_2 kg^{-2} h^{-1} at the same set of temperature regimes (Fig 8).

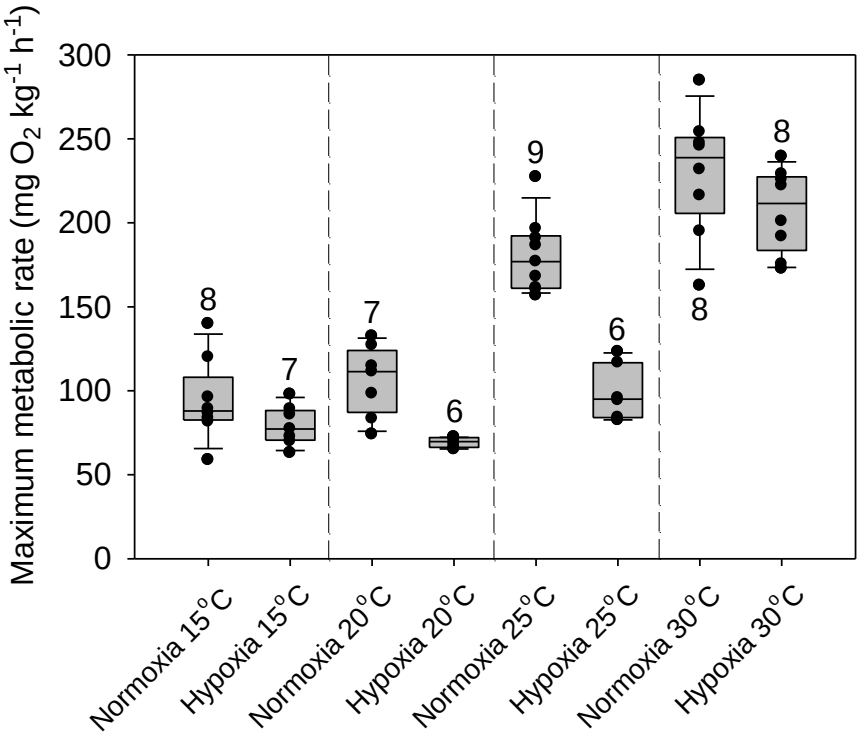
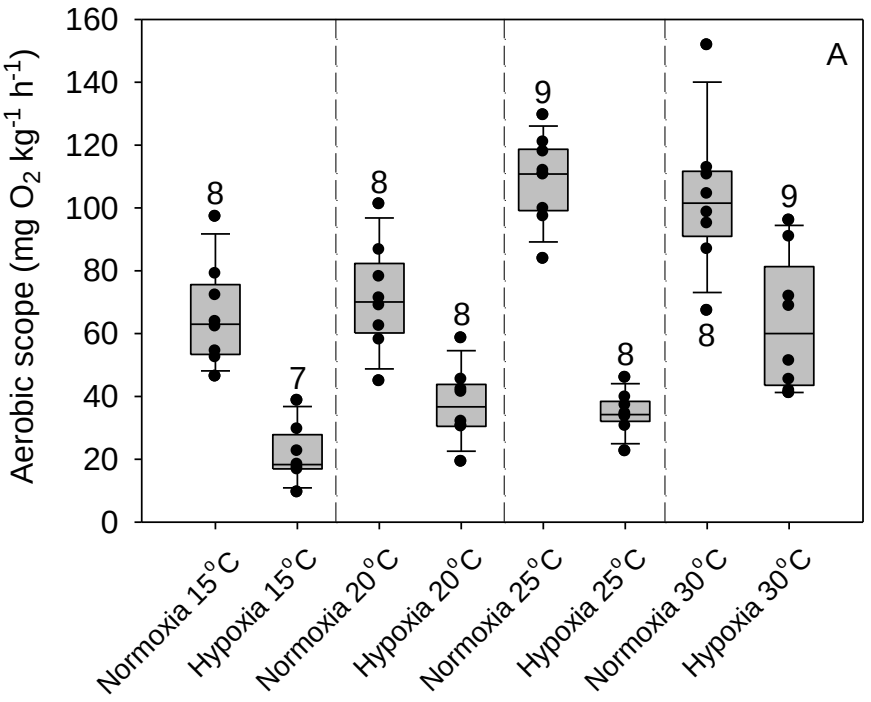


Figure 8 Maximum metabolic rate (MMR) of individual lionfish under normoxic and mildly hypoxic conditions across four temperature regimes (15, 20, 25, and 30℃). Median values are represented by the horizontal center line within each box. Numerical values above the error bars signify (n) individuals tested for the respective species. The grey rectangular boxes span the first quartile to the third quartile with the whiskers showing the minimum and maximum values excluding outliers. Outliers are data points above or below the whiskers for each box. Units are in mg O_2 kg^{-1} h^{-1}

Median metabolic scopes of lionfish increased with temperature and were found to be significantly different across all temperature regimes except between 25℃ and 30℃, as well as 15℃ and 20℃ (Fig. 9a). Q_{10} values calculated from metabolic-scope values were 2.4 between 15℃ and 25℃ and 1.94 between 20℃ and 30℃ indicating that metabolic-scope more than doubles with increases in temperature at the lower end of the lionfish tolerance range and the rate of increase slows at higher temperatures. Hypoxia

severely limited the metabolic-scope of lionfish from 63.0 ±5.8, 70.1 ±6.2, 110.7 ±5.2, 101.5 ±8.6 under normoxic conditions to 18.3 ±3.4, 36.7.0 ±4.2, 34.2 ±2.4, 60.0 ±7.7 at each temperature regime respectively (Fig. 9a). Relative aerobic scopes (ratio of MMR/SMR) magnify these significant differences across all temperatures between the limiting effects of hypoxia on lionfish metabolic scope (Fig. 9b). Median factorial scopes at 15, 20, 25, and 30℃ under normoxic conditions were 3.29 ±0.23, 2.78 ±0.27, 2.68 ±0.15, 1.80 ±0.06 respectively and 1.34 ±0.11, 1.61 ±0.38, 1.55 ±0.09, 1.44 ±0.07 under hypoxic conditions respectively Fig. 9b). The decrease in factorial scope followed the decrease in MMR under hypoxia and consequently, reduced metabolic-scope.



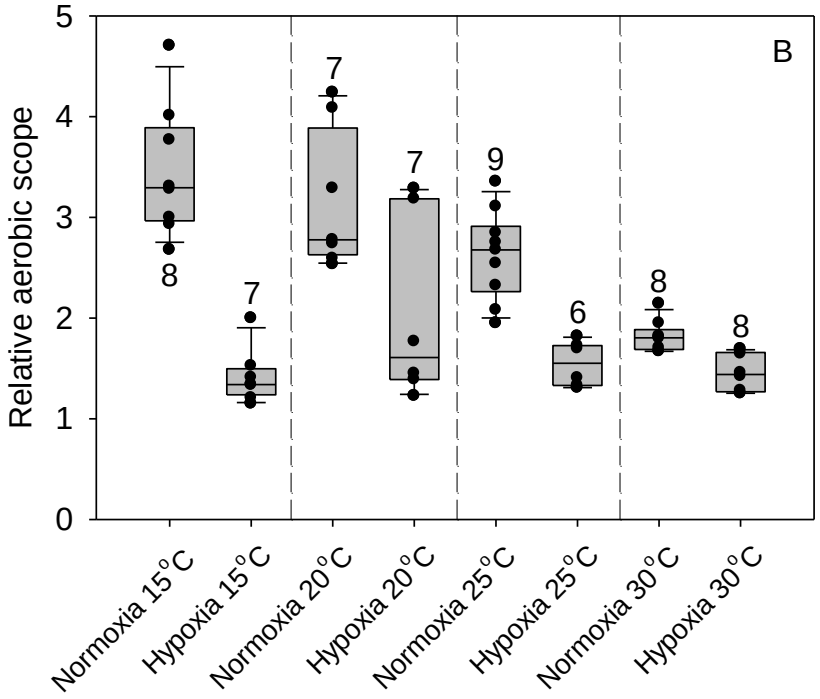


Figure 9 (a) Metabolic-scopes (MMR-SMR) **(b)** Relative metabolic scope (MMR/SMR) of individual lionfish at various temperatures under normoxic and hypoxic conditions. Box and whisker plots were constructed using SigmaPlot 11 and median values are represented by the horizontal center line within each box. Sample size (n) for the respective condition is indicated by the numerical value above the maximum error bar. Outliers are shown as dark circles extending past the whiskers for each box. The grey rectangular boxes span the first quartile to the third quartile with the whiskers showing the minimum and maximum values excluding outliers. Metabolic scope measurements are displayed in oxygen content mg O_2 kg^{-1} h^{-1}

Critical Oxygen Saturation Level

Median S_{crit} values were 33% (±1), 39% (±1), 46% (±1), 54% (±1) respectively (Fig. 10a). Measurements were taken from fully recovered fish at a stabilized SMR. Critical oxygen levels are expressed as S_{crit} and C_{crit} to make these results comparable to a larger body of literature and similar conclusions can be made even given that equal levels of air saturation represent lower levels of oxygen content at higher temperatures. S_{crit} and

C_{crit} were significantly different at all temperatures with higher temperatures yielding higher critical oxygen values (Fig. 10a and b). Median C_{crit} values at 15, 20, 25, and 30℃ were 3.0 ±0.1, 3.1 ±0.1, 3.4 ± 0.1, and 3.6 ±0.2 respectively (Fig. 10b).

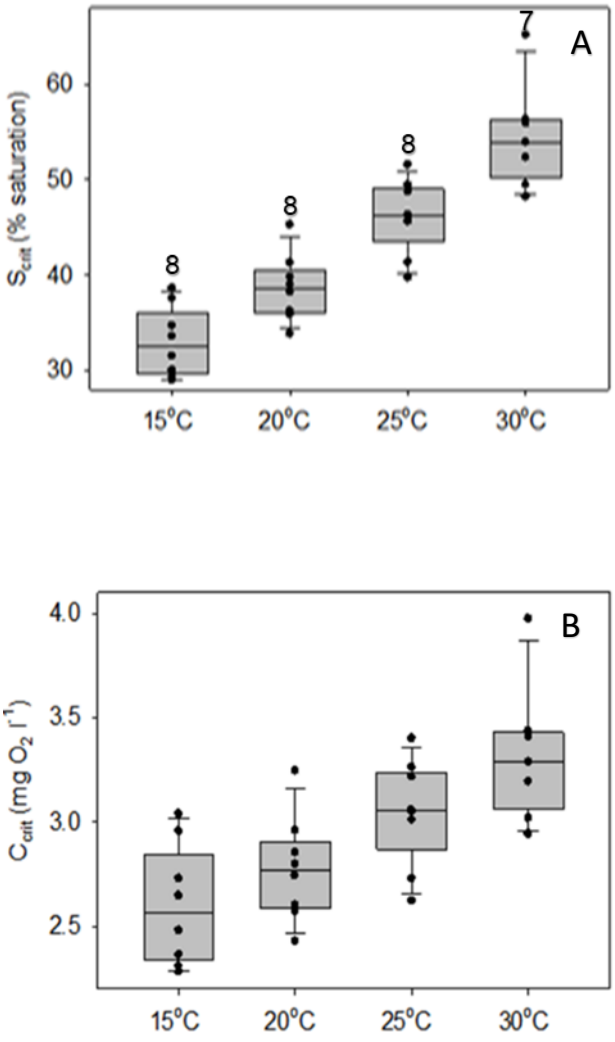


Figure 10 (a) Critical oxygen levels expressed as % air saturation (S_{crit}) and **(b)** Critical oxygen levels expressed at oxygen content (mg O_2 l^{-1}) of seven individual lionfish at four different temperature regimes. S_{crit} values were calculated using piece-wise, segmented linear regressions (SegReg program, freely available at http://www.waterlog.info/segreg.htm) to determine the air saturation at which lionfish could no longer maintain routine metabolic rate. C_{crit} values were converted from S_{crit}

values using a freely available online calculator at https://www.loligosystems.com/convert-oxygen-units

Discussion

Luminous Sensitivity

Lionfish median luminous sensitivitiy (0.24 log cd m^{-2}) (Fig. 2), quantified by photopic K_{50} values was left-shifted compared to those of striped bass (1.84), bluefish (0.93), and cobia (1.00) (Horodysky et al., 2010) and most similar to summer flounder (0.19) indicating a heightened sensitivity in dimmer environments (Fig. 3). Although lionfish are more active hunters than the majority of scorpaenids, their heightened luminous sensitivities influence similar feeding ecologies compared with summer flounder, in that they prefer benthic, ambush-based feeding. Therefore dimly-lit or turbid environments within estuaries will not prevent lionfish from detecting targets in close proximity especially in conjunction with their other sensory adaptations.

Dynamic ranges, which are quantified by range of luminous sensitivity, were drastically reduced in lionfish (1.98 log units) compared with all of the estuarine fishes observed in Horodysky et al. 2010 (Fig. 3). Because estuaries are some of the most photodynamic environments on the planet, lionfish will be limited to sheltered or deeper habitats that do not experience as much luminous fluctuation or reduce activity levels until more optimal conditions occur.

This specificity may have been influenced by temporal feeding patterns consistent with lionfish crepuscular foraging behavior or their preference for greater depths (>30 m) (Whitfield et al., 2014), where lighting conditions are consistent and constantly limited. Fluctuating light intensities are common occurrences within estuarine ecosystems due to a variety of influences including sedimentation, nutrient load, tidal movements, etc. Lionfish may not be able to achromatically contrast within regions of estuaries that experience rapid and drastic changes in light characteristics; especially those with periodic, large swings in turbidity. Many estuarine species possess broader dynamic ranges to compensate for such changes, thereby capitalizing when prey is abundant under

constantly varying ambient light intensities. However, lionfish are better adapted to visualizing in low-light environments during daylight conditions, filling a visual niche not observed by the other estuarine piscivores in Horodysky (2008, 2010) with the exception of striped bass. Accommodating for the lower light levels associated with increased turbidity in estuaries would be an easy task for the lionfish visual system, rendering them just as effective as other estuarine predators given time to acclimate to these light conditions. Decreased light levels will not be a barrier to lionfish as effective visual predators and will likely only enhance their competitive ability given their heightened luminous sensitivities. As lionfish continue to expand into inshore ecosystems, they will likely associate with deeper habitats, man-made structures, and other abiotic/biotic features limiting them to more light-stable conditions.

Flicker Fusion Frequency

Lionfish median flicker fusion frequencies at I_{25} (35.7 Hz), representing ambient light levels, were lower than those of striped bass, bluefish, and cobia (56.3, 44.1, and 58.7 Hz respectively) and similar to those of summer flounder (36.9) (Fig. 4) (Horodysky et al., 2010). Under ambient estuarine light conditions, the visual acuity of lionfish would be at a strong disadvantage, taking a much longer time for the retina to receive enough photons to produce a sharp, resolute image. However, as previously seen with the previous K_{50} values, lionfish possess heightened sensitivity at the expense of visual acuity under dimmer light, and presumably optimize this modality under such conditions.

Conversely, lionfish displayed higher median FFF values (85 ±7 Hz) than those of the highly visual estuarine piscivores at I_{max} (100% *V/LogI*) striped bass (76), bluefish (56.6), and cobia (61)) (Fig. 4) (Horodysky et al., 2010). In shallow, clear, brightly-lit environments, such as the (sub)-tropical reefs of the Indo-Pacific, lionfish maximize visual acuity to track fast-moving prey through 3-D, structurally complex, coral-reef ecosystems. Lionfish prefer similar conditions within estuaries since it provides more energetically-favorable, visual-ambush based foraging versus search and encounter-rate foraging under lower light conditions (Horodysky et al., 2016). Shallow areas of estuaries as well as associated rivers and tributaries buffered by large mangrove or saltmarsh

wetlands are common areas for visual predators to forage during flood tides due to increased water clarity.

Lionfish optimize sensitivity in temporal or location specific low-light environments, and rely on the expediently quick, precise, resolving capabilities of their visual systems under high indecent light. Therefore, neither low light nor brightly lit conditions would pose as substantial enough visual barriers to impede lionfish from effectively foraging within estuaries. This evolved visual ecology assumes a generalist approach in that even though lionfish visual systems may appear fairly rigid, they are previously adapted to meet the respective achromatic properties and resolution demands from a wide-variety of marine ecosystems.

Spectral Sensitivity

Lionfish possessed spectral sensitivities similar to those of coastal estuarine piscivores spanning a total of 290 nm with the highest median sensitivity at 490 nm, consistent with blue-green color wavelengths (Fig. 5) (Horodysky et al., 2010). Both lionfish and many estuarine fishes are dichromats, possessing two visual pigments arranged in a specific fashion to both optimize sensitivity in low-light environments and contrast prey targets against the dominant background (cite). Visual pigment estimations within the lionfish eye were based on a comparison of rhodopsin templates fitted to the ERG data and produced a short-wave pigment at 434 ±2.6 nm (blue), an intermediate pigment at 512 ±1.0 nm (green), and a beta band at 381 nm (Fig. 5). Estuarine fishes possess comparable pigment orientations with short-wavelength pigments ranging from 440 to 460 nm and intermediate pigment values of 520 to 540 nm (Lythgoe and Partridge, 1991; Lythgoe et al., 1994; Horodysky et al., 2008; 2010). Species-specific pigment mechanisms were further validated through spectrophotometry of lionfish ocular media. The relative transmittance of both the cornea and lens of lionfish was highest in the blue-green wavelengths confirming the pigment estimations by the rhodopsin template (Fig. 6).

Carroll et al., (2018 M.S. Thesis) recently observed that neither lighting condition nor time influence lionfish spectral sensitivities indicating that visual plasticity may only be limited to specific life history stages. This provides contrast to other fishes such as

tarpon that adjust their retinal sensitivity based on their circadian rhythms (Kopperud and Grace 2017). Since lionfish do not appear to exhibit short-term retinal plasticity, the blue-green wavelength sensitivities are an excellent ubiquitous adaptation for deeper, mesophotic waters between 30-100 m as well as coastal environments (Horodysky et al., 2008). The similarities between the spectral sensitivities of lionfish and other estuarine fishes suggests that lionfish should be well suited for the chromatic properties of estuarine environments and therefore will be able to effectively differentiate prey-targets within them.

Ecological Implications

Overall, this study confirms that lionfish have the visual capacity to continue to migrate throughout areas within Atlantic and Gulf of Mexico inshore estuaries and will not be inhibited by the photo-light characteristics within them. This is an unnerving prospect, considering lionfish have already been shown to reduce recruitment of native fishes on coral reefs and salinity does not appear to be a barrier to their movement patterns (Albins and Hixon 2008; Jud et al., 2011). Lionfish have evolved to live in many habitats encompassing a vast variety of light and spectral compositions and take a "one size fits all", approach to their generalist visual ecology. As they move further inshore, lionfish can be expected to prefer clear, brightly-lit areas but will likely aggregate to habitats where light-intensity fluctuations are minimized to establish more consistent, favorable lighting regimes. Such areas include small tidal rivers and tributaries buffered by large areas of coastal wetlands, man-made structures (i.e. bridges, docks, etc.), and parts of deeper salt wedge estuaries like the Chesapeake Bay. Other Mid-Atlantic estuaries like the Cape Fear River estuary in North Carolina are partially mixed estuaries, dominated by strong tidal surges associated with discrete haloclines. Lionfish would have potential access to an extensive network of stable, buffered watershed that experiences occasional saltwater surges up to 112 km inshore (Geise et al., 1985 Hydrology of major estuaries and sounds of NC) if the current temperature ranges did not prevent them from doing so. The balancing act between heightened luminous sensitivity at lower light levels and exceptional visual acuity at higher light levels provide lionfish with a visual ecology suitable for a wide variety of light environments, including many habitats within inshore

estuarine ecosystems. Additionally, this study illustrates that lionfish adopt both the contrast and sensitivity hypotheses when forming images in the marine environment. Because lionfish possess blue-green pigment wavelengths that dominate within coastal and estuarine systems, they possess the means to match both the dominant wavelength to maximize photon capture, and another primary color wavelength to optimize contrast sensitivity thereby increasing perception. The lionfish visual system was similar, and occasionally excelled interspecifically compared to the specialized visual characteristics of the estuarine piscivores herein (Summer flounder, striped bass, bluefish, cobia) and should not be taken lightly in implications for increased inshore invasive success. However, many other environmental parameters must be taken into account in conjunction with their mechanistic, physiological influences on ecology to answer the question; What are the comprehensive physiological limits of lionfish sp. preventing further inshore invasion of western Atlantic/Gulf of Mexico inshore estuaries?

Metabolic rates

Lionfish SMRs from this study agree with previously observed values recorded by Cerino et al., (2013). At 30℃, the SMR of lionfish from the current study was (131 ±7 mg O_2 kg^{-1} h^{-1}) (Fig. 7) while Cerino et al., (2013)'s weight-specific least squares model approximated an SMR of (125 mg O_2 kg^{-1} h^{-1}) at 29.1 ℃ of fish with similar masses to animals used in this study. Lionfish SMRs scaled similarly, but on the higher end of the continuum of tropical/sub-tropical scorpaenids. SMRs of the golden scorpionfish, *Parascorpaena aurita*, were reported to be 68.9 mg O_2 kg^{-1} h^{-1} at 20℃ (Zimmerman and Kunzmann 2001), meanwhile lionfish SMRs recorded at the same temperature in the current study were 70.07 ±6.17 (Fig. 7). Golden scorpionfish may be quality candidates of general interspecific comparisons in this matter since adults are generally the same size range as the experimental lionfish used in this study. Foraging behaviors of lionfish and other scorpaenids reflect the metabolic similarities of these species and as a result, their similar ecologies. Many feeding ecologies of scorpionfishes display cryptic lie and wait behaviors, or sluggish, passive swimming followed by burst-ambush predation. This "aggressive patience" feeding strategy is highly influenced by relatively low SMRs, keeping energetic and oxygen demands minimal when prey is not abundant, but capitalizing on short bouts of explosive burst-swimming with subsequent quick recovery

periods and expedited digestion when prey items are abundant. Lionfish appear to be more active than the majority of scorpionfish species which is illustrated by their elevated metabolic scope but still remain on the sluggish side of the metabolic spectrum of fishes.

MMRs of lionfish were heavily suppressed at the lower end of their thermal range (88.0 ±8.8) mg O_2 kg^{-2} h^{-1} at 15℃, but rapidly increased to (238.8 ±13.4) mg O_2 kg^{-2} h^{-1} at 30℃ as their optimal thermal temperature was reached. The Q_{10} value (3.4) between 20 and 30℃ demonstrates this immense jump in MMR values of lionfish with severe implications for future global temperature trends. The rate of increase of MMR will begin to slow and eventually plateau when the cardio-respiratory system can no longer keep up with the metabolic oxygen demands required at increased temperatures. Hypoxia only suppressed MMR at intermediate temperatures, 20 and 25℃ and had little to no effect on MMR at the lowest and highest temperature regimes. The former interaction was exclusively influenced by temperature acting as the controlling effect on MMR rather then the limiting effects of low oxygen conditions which supports the minimum winter temperature boundaries proposed by Whitfield et al., (2014). The negligible effect of hypoxia on MMR at 30℃ is likely attributed to this regime being centered around the optimal thermal temperature of lionfish for fitness, and at relatively hypoxic levels (15-20% above S_{crit}) does not appear to negatively impact performance as suboptimal temperature ranges do. Temperatures above this optimal temperature regime will likely drastically reduce MMR under mild hypoxic levels but, further insight is needed into the relationship of hypoxia on lionfish metabolism at temperatures above 30℃.

Investigating the interactive controlling and limiting effects of temperature and oxygen on metabolic scope allowed us insights as to whether lionfish have the hypoxia tolerances that would make them capable of extensive inshore invasion. Lionfish median metabolic-scopes spanned from (63 ±6) mg O_2 kg^{-1} h^{-1} at 15℃ to (101 ± 9) mg O_2 kg^{-1} h^{-1} at 30℃ (Fig. 9a) and were heavily dependent on temperature and oxygen level. Metabolic scopes divided into two significantly different groups across the four temperatures: 15℃ and 20℃ represented a suppressed temperature-metabolic scope regime versus increased metabolic scopes that plateau at 25℃ 30℃ (Fig 9a.). The higher

plateau suggests an optimal temperature range for the aerobic scope of lionfish which is further supported by findings in Barker et al., (2014) and Cerino et al., (2010). Barker et al., (2014) found that the final temperature preferendum of lionfish with gradual acclimation is (28.7 ±1) and Cerino et al., (2010) observed that the optimal temperature for prey consumption is 29.8℃. Q_{10} values further illustrate the dynamic confines of metabolic scope indicating that metabolic scope approximately doubles with increases in temperature of 10℃, but the ratio of increase rapidly attenuates at higher temperatures past 25℃. Cerino et al., (2014) found respiration Q_{10} values of lionfish were 2.08 comparing similarly with the doubling of metabolic rate with temperature observed in our study. Further insight is needed into how temperatures above 30℃ may affect lionfish metabolic scope as the upper thermal tolerance limit established by Barker et al., (2014) was found to be 40℃ and lionfish tend to prefer temperatures closer to their CT_{max} rather than their CT_{min}.

Median metabolic-scopes were severely limited by hypoxia across all temperatures as MMRs at each temperature regime were significantly depressed. The factorial decreases in metabolic scope between normoxia and hypoxia definitively illustrate this limitation. Under normoxic conditions, factorial scopes were 3.29 ±0.23, 2.78 ±0.27, 2.68 ±0.15, and 1.8 ±0.05 as opposed to 1.34 ±0.11, 1.61 ±0.38, 1.55 ±0.09, and 1.44 ±0.07. at 15, 20, 25, and 30℃ respectively (Fig. 9b). These findings suggest that lionfish metabolic rates are reduced to roughly 50% at mildly hypoxic oxygen levels, 15-20% above their S_{crit} value, with the exception of moderately higher temperatures (30℃). Hypoxia may not restrict metabolic scope as much at these temperatures ~ (28-32℃) since they are within the optimal temperature range of lionfish based on findings in the current study previously conducted work by Cerino et al., (2013) and Barker et al., (2014). As temperatures exceed this range, metabolic scopes will rapidly decline due to decreased oxygen solubility at higher temperatures and failure of the cardio-respiratory system to meet the associated oxygen demands. Even though lionfish would be able to survive over the long term at oxygen levels 15-25% above their S_{crit}, prolonged exposure would result in a number of sub-lethal effects impacting fecundity, predation and digestion rates, growth rate, etc. (Brill et al., 2015).

Critical Oxygen Saturation Levels

Lionfish resemble other teleost fishes in that they are oxygen regulators, possessing the physiological ability to maintain a constant metabolic rate until the critical oxygen level is reached and rates of oxygen delivery from the cardio-respiratory system can no longer maintain SMR (Brill et al., 2015). Critical oxygen values of lionfish (S_{crit}/C_{crit}) spanning the four temperatures (Fig. 10a and 10b) indicated that lionfish are very hypoxia intolerant in comparison to other estuarine fishes. Spot and croaker, common estuarine, scianid fishes that range throughout the Atlantic coast, possess S_{crit} values almost half those of lionfish allowing the former species to compensate for the episodic hypoxia in estuaries limiting their metabolic scopes (Marcek et al., 2018). S_{crit} values of Atlantic croaker, spot, and lionfish at 15℃ were (17.6%, 17.2%, and 32.6 ±1.3%) respectively and (34.8%, 29.0%, and 53.9 ±1.36%) at 30℃ respectively (Marcek et al., 2018). Even more actively-swimming estuarine fishes with higher oxygen demands, such as striped bass (25%), have lower S_{crit} values than lionfish (38.6 ±1.26%) (Fig 10a) at similar temperatures (20℃) (Lapointe et al., 2014).

During the summer months, the hypoxic discharge of major Mid-Atlantic rivers and tributaries would contain the lionfish invasion closer to the ocean-side, mouth of estuaries such as the Cape Fear river and Pimilico sound estuaries in North Carolina, where associated river mouths emanate dissolved oxygen levels approximately (~3.5mg/L) or less (Giese et al., 1985). As water temperatures cool, lionfish will likely move further inshore, as oxygen levels return to close 100% saturation in these regions. However, current annual Winter temperatures for mid-Atlantic estuaries would not allow for permanent establishment of year-round populations and would thus exclude lionfish from the majority of associated inshore rivers and tributaries. Caution must be advised as climate change may expand the physiological limit for further inshore expansion since temperatures and hypoxia in the winter months will no longer be barriers to lionfish accessing a substantial part of the mid-Atlantic watershed (Whitfield et al., 2014). Gulf of Mexico estuarine systems are frequently characterized as some of the more hypoxic of estuarine environments, with extensive dissolved oxygen levels remaining below 2 mg/L greater than 20% of the time (Summers et al., 1997). South Atlantic estuaries also

experience drastic seasonal decreases in dissolved oxygen below lionfish S_{crit} values (Fig. 10a and 10b) primarily from agricultural runoff and urbanized land use contributing to eutrophication (Robbins and Lisle 2018). It is unlikely that lionfish will be able to extensively exploit the majority of Gulf of Mexico/South Atlantic estuaries in the Summer months, as the oxygen levels are far below that of their S_{crit}/C_{cirt} values (Fig. 10a and 10b) and hypoxic/anoxic zones are only predicted to expand in these regions (Summers et al., 1997). As water temperatures cool, hypoxia may no longer be a barrier to lionfish in more productive estuaries with higher mean dissolved oxygen levels, and winter temperatures will not be low enough to limit their inshore spread. This is evidenced by lionfish being observed up to 4 km inshore within the Loxahatchee River estuary (Jud et al., 2011). In summary, because minimum dissolved oxygen levels are below that of the S_{crit} value for lionfish a significant period of the year, the extent of invasion into these estuarine systems will be highly limited to and permanent year-round establishment unlikely.

It is clear that through both interspecific comparisons to hypoxia-tolerant estuarine fishes and because S_{crit} values represent the lowest oxygen level that allows long-term survival, invasive expansion of lionfish into inshore estuarine systems will be highly limited. Oxygen levels below S_{Crit}/C_{crit} would extend digestion rates, inhibit feeding and movement, and reduce overall physiological performance unless individuals are able to migrate to another location or withstand the conditions until oxygen levels return to normal. Lionfish appear to be highly sensitive to hypoxia and may actively avoid lower dissolved oxygen levels entirely. During occasional periods of S_{crit} trials where the oxygen level was being reduced (~10%) well above the S_{crit} value, some subjects would begin moving rapidly back and forth within the chamber seemingly displaying avoidance behavior. Behavioral responses of lionfish (avoidance vs. conserve energy) to low oxygen are influenced by their metabolic rate, therefore, lionfish that have elevated metabolic rates from exhaustive exercise, digestion, or disease would exhibit behavioral responses at even higher oxygen levels. Conversely, lionfish are highly dependent upon the rate of oxygen decline due to their sensitivities to dissolved oxygen level (Brill et al., 2015). Further research is needed examining the sensitivity and

behavioral responses of lionfish to low dissolved oxygen before any additional ecophysiological insights can be made on this matter.

Ecological Implications II

Lionfish metabolic scopes and their interaction with temperature are consistent with general teleost physiology trends in the literature, and the previous studies investigating SMRs, thermal preferences, and optimal temperatures for aerobic fitness also solidify the validity of this study's findings (Cerino et al., 2013; Barker et al., 2014). More importantly, the findings of the current study support the original hypothesis: that low dissolved oxygen will pose as a significant barrier limiting the invasive spread of lionfish into Atlantic and Gulf of Mexico inshore estuaries. Estuarine fishes have adapted highly-specialized, hypoxia-induced physiologies that include increased mass-specific gill surface area, increased hemoglobin-O_2 binding affinities, and induced metabolic suppression (Mandic et al., 2008; Richards et al., 2009). These hypoxia-tolerant, physiological adaptations are reflected in the lower S_{crit} values of estuarine species in contrast to lionfish that do not possess such adaptations as low oxygen is not commonly associated with the environments they naturally inhabit. In addition, intertidal areas of estuarine-associated rivers and tributaries experience oxygen levels substantially below that of lionfish S_{crit} values during the Summer months, therefore, year-round inshore establishment of lionfish populations will be unlikely in these areas (Geise et al., 1985; Summers et al., 1997, Mallin et al., 2014). The region at greatest risk for invasion would be South Atlantic estuaries, since these ecosystems do not have the low temperatures of mid-Atlantic estuaries thus preventing over-Winter establishment and are not as characteristically hypoxic as Gulf of Mexico estuaries (Summers et al., 1997). Still, lionfish appear to be relatively hypoxia sensitive and would likely avoid low-oxygen environments as soon as they are detected. Furthermore, water quality of South Atlantic estuaries has rapidly been degraded due to eutrophication from agricultural runoff and heavily concentrated urbanization contributing to low oxygen levels (Summers et al., 1997). Even if lionfish were capable of successfully migrating further inshore, their reduced metabolic scopes under low oxygen conditions would render them either physiologically handicapped or force them to relocate to a habitat with more favorable

conditions. We therefore conclude that lionfish will not pose a threat to the vast majority of estuarine ecosystems characterized by episodic hypoxia due to their heavily constrained metabolic scope and low physiological tolerances to low oxygen environments.

Conclusion

This study advances the understanding of the ecophysiological mechanisms underlying the visual ecologies, metabolic performance, and hypoxia tolerances of invasive lionfish to determine the limits of inshore invasion. We have concluded that seasonal changes in temperature-dissolved oxygen levels consistent with Atlantic/Gulf of Mexico inshore estuaries not only exceed the physiological tolerances of lionfish but also constrain metabolic scope up to 50% at sub-lethal levels, severely limiting their seasonal infiltration into associated rivers and tributaries. Furthermore, we conclude that lionfish possess a generalist visual system that can satisfy the visual demands from a large variety of environments including those of estuarine ecosystems. Interspecific comparisons illustrate the consistencies among visual characteristics between lionfish and estuarine piscivores. In summary, lionfish should not pose a significant threat to commercially and ecologically important species that utilize inshore watersheds as nursery areas. However, caution should still be advised as lionfish appear to be visually capable of foraging effectively within estuaries and may capitalize on specific temporal and spatial scales that provide suitable habitat quality and abundance of prey items.

For this reason, further research modeling the predicated invasion of lionfish into sensitive inshore ecosystems is needed. Salinity is also a key factor that heavily influences metabolic rates of fishes and was maintained at 20 ppt within this study for purposes of resembling “estuarine conditions”. However, salinity levels fluctuate over vast, wide ranges within estuaries and the osmoregulatory tolerances and abilities of lionfish remain largely unstudied. Ecological insights derived from involved physiological mechanisms, such as those in this study, are invaluable for forecasting current and future population effects of lionfish in response to varying environmental conditions. Such advances would provide fisheries management agencies with enhanced control measures and allocation of resources to combat the expected exacerbated spread

of these species, especially with the future increase in suitable thermal habitat (Whitfield et al., 2014). The future fundamental range of lionfish throughout the Western Atlantic and Gulf of Mexico is an unnerving prospect, but by providing novel ecophysiological explanations on how lionfish relate to their environment, great accomplishments can be achieved in effectively controlling these species and furthermore, minimizing the loss of valuable living resources and biodiversity.

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