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**LIGHT DEPENDENT FEEDING AND PHOTOGRAMMETRIC
DETERMINATION OF MOVEMENT SPEED OF PTEROIS VOLITANS
AND PTEROIS MILES IN THE FLORIDA KEYS**

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**LIGHT DEPENDENT FEEDING AND PHOTOGRAMMETRIC
DETERMINATION OF MOVEMENT SPEED OF *PTEROIS*
VOLITANS AND *PTEROIS MILES* IN THE FLORIDA KEYS**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Oceanography and Coastal Sciences

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Neal Kolonay
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TABLE OF CONTENTS

Acknowledgements.....	ii
Abstract.....	iv
General Introduction.....	1
Chapter 1. Laboratory Based Light Availability Experiments.....	4
1.1. Introduction.....	4
1.2. Methods.....	8
1.3. Results.....	13
1.4. Discussion.....	18
Chapter 2. Unbaited Stereoscopic Video Traps.....	24
2.1. Introduction.....	24
2.2. Methods.....	29
2.3. Results.....	38
2.4. Discussion.....	43
References.....	49
Vita.....	58

Abstract

Invasive species are known to cause economic and ecological damage worldwide, with many aquatic and terrestrial species extending outside their range. Invasive lionfish, *Pterois volitans* and *Pterois miles*, are currently some of the most destructive marine species, being released into non-native Atlantic waters, gaining a foothold, and expanding at explosive rates. While attempts have been made to cull their populations through various methods, primarily spearfishing, their populations continue to grow and their ranges expand showing a need for a deeper understanding of their ecology to more effectively model and target them. In this work, two avenues of lionfish ecology which are currently poorly understood will be explored; differences in feeding behavior under differing light conditions and in-situ movement speeds of lionfish living in shallow reef environments. As lionfish populations expand geographically, their movement speeds will be an important factor in understanding where individual fish may spread and what impacts they may have to native fish populations. In addition, their proliferation has been documented to include much deeper habitats than what is typically considered in reef ecology, and as such experiments were run to determine what difference, if any, in feeding rates occurred under different light conditions. In-situ movement speed and behavioral categorization were completed using stereoscopic systems and photogrammetric analysis. Results of the laboratory experiments suggest that differences in lionfish feeding do occur under different light conditions.

General Introduction

Lionfishes, both *Pterois volitans* and *P. miles* (hereafter referred to as lionfish), have been an invasive species in the waters of the Western Atlantic and the Caribbean for over 30 years, primarily due to their fast reproduction and lack of local predators (Luiz et al. 2021). Native to the Indo-Pacific, they were most likely introduced by aquarium keepers off the coast of Florida and their invaded range now reaches from New York, through the Gulf of Mexico, the Caribbean, and even down to Brazil (Ferreira et al. 2015). High densities of lionfish can cause a loss of over 97% of prey-sized native fishes in just 7 weeks on reefs where they are found due to high feeding rates (Benkwitt 2014) and can reduce recruitment of native species by an average of 79% due to predation on juvenile or larval fishes (Albins and Hixon 2008). This can cause a loss of biodiversity, as well as decrease the abundance of adult predators such as snapper and groupers, both due to predation on juveniles as well as competition for the prey that these larger piscivores target (Morris and Whitfield 2009).

Invasive lionfish influence a range of both ecological and economical systems (Malpica-Cruz 2017) and are expected to continue to do so in the future as their populations grow and disperse further (Albins and Hixon 2013). Lionfish have a diverse diet that includes grouper, goatfish, parrotfish, blennies, damselfishes, crustaceans, mollusks and more (Muñoz et al. 2011). A decrease in the abundance of fish and invertebrates on a reef can result in a decreased overall reef health (Díaz-Pérez et al. 2016), and predation on important herbivorous grazers can allow macroalgae to proliferate and outcompete corals (Mumby 2006). An estimated \$35.8 billion USD is generated annually by tourism related directly to coral reefs and lionfish can impact industries related to reef-associated tourism and fishing (Spalding et al. 2017). These effects may

begin to heavily impact both tourism and the fishery industries, which are closely tied to reef conservation and health.

Lionfish have been successful in their invaded range for several reasons. One major reason is that lionfish have very few organisms willing to prey upon them in the invaded range, and the presence of potential piscivorous predators does not significantly change lionfish density in that range (Cure et al. 2014). Two other major contributors to their success are their generalist and indiscriminate diet and their successful hunting (Muñoz et al. 2011, Albins and Hixon 2008, Morris and Akins 2009, Côté et al. 2013). These fish are able to consume large amounts of food in a short time, with their stomachs extending up to 30 times the original size (Morris and Whitfield 2009) and consuming an average of 14.6 g day^{-1} as adults of 300–500 g body weight (Fishelson 1997).

Lionfish employ several behaviors to increase hunting success. Primary among these include crepuscular hunting (hunting when light levels are low at dawn and dusk) making it harder for prey species to avoid them, as well as “blowing” behavior (ejecting jets of water from their mouth) to disorient their prey (Cure et al. 2012). This blowing behavior is greatly reduced in the Atlantic in comparison to their native range, suggesting a certain level of naïveté in their target prey which allows an even higher hunting success (Cure et al. 2012). In addition, they are able to fast for long periods with minor effects, losing only 5–16% of body weight after 3 months of fasting (Fishelson 1997), which allows them to continue surviving when local food levels begin to deplete, and gives them sufficient time to relocate.

While the majority of previous studies have focused on shallow coral reefs, lionfish have been found in mesophotic reefs as well, defined as reefs with low light availability between 30 and 200 meters depth (Andradi-Brown et al. 2017, Nuttall et al. 2014) with sightings past 300

meters in some areas (Gress et al. 2017). The lionfish found on these reefs are of a larger average size, show a difference in gender ratios in favor of females, show a much higher gonad to body weight ratio, and are more mature in comparison with shallow reefs (Andradi-Brown et al. 2017). Mesophotic lionfish stomachs were found to have similar fullness in both ecosystems, suggesting that lionfish in mesophotic systems are obtaining sufficient food despite the lower light conditions (Andradi-Brown et al. 2017). The mesophotic reefs within their invaded range represent ecosystems with different physical conditions than shallow reefs, and these differences could influence lionfish interspecific and intraspecific interactions within mesophotic reefs.

Current understanding of lionfish growth as it relates to temperature, respiration, and excretion can inform the the maximum potential of lionfish growth (Cerino et al. 2013); however, there are many aspects of lionfish foraging and behavior that can enhance our understanding of how lionfish individuals grow and populations proliferate. A new bioenergetics-foraging model is being created by the Marine and Community Ecology laboratory at Louisiana State University to more accurately model how lionfish hunt and utilize energy. This model will be used to influence future conservation practices by giving a representation of growth under different conditions. In-Situ swimming speed of different behaviors and light dependent feeding are two major factors that have not been well studied in lionfish, but which could provide valuable insight to their foraging behavior. Swimming speed can be used to quantify how much habitat area a lionfish may cover in a day, and their success at feeding under different light conditions could be an important factor in understanding their survival and growth in mesophotic areas where they are now found. Understanding lionfish foraging behavior would be useful in calculating lionfish growth rate potential, mapping key habitat for lionfish, and determining the best course of action for conservation efforts moving forward.

Chapter 1. Laboratory Based Light Availability Experiments

1.1. Introduction

Lionfish are a widespread invasive species, with detrimental effects on native species. High growth rates, high fecundity, frequent spawning, indiscriminate feeding, and few predators in the invaded range allow lionfish to be effective invaders (Albins and Hixon 2016). Healthy and fecund populations of lionfish are also abundant on mesophotic reefs which are not commonly studied (Andradi-Brown et al. 2017, Gress et al. 2017). Having data on how successful lionfish are on these mesophotic reefs would be beneficial for conservation practices, allowing more accurate targeting of populations.

Mesophotic reefs vary in their definitions, but there are typically two major differences used to distinguish them from shallow reefs. These differences include the makeup of photosynthetic coral communities and in light levels, and these are often closely related (Hinderstein et al. 2010, Laverick et al. 2017, Tamir et al. 2019). The light conditions on any reef will vary due to several environmental factors such as turbidity, salinity, changes in solar irradiance, and water both scatters and absorbs light as depth increases (Anthony et al. 2004, Davies-Colley and Smith 2001). Light attenuation with depth changes with both the intensity of light and the dominant wavelengths of the light, resulting in light composed of only a few wavelengths in the blue portion of the visible light spectrum reaching deep water in the 40-200m range (Sullivan 1963, Muaddi and Jamal 1991, Pegau et al. 1997, Lee et al. 1998).

Crepuscular activity is typically defined as occurring during two periods in the diel cycle when light conditions are actively changing, dusk and dawn (Helfman 1986, Green et al. 2011). Lionfish are active primarily during these crepuscular times for hunting, mating, and general

movement (Fishelson 1975, Green et al. 2011, Cure et al. 2012). Although lionfish primarily hunt during crepuscular times, several studies have noted their propensity for increased hunting behaviors during days that were overcast, or when cloud cover increased (Côté and Maljković 2010, Green et al. 2011, Cure et al. 2012). The exact reason for this change in behavior is not clear, but it may suggest that they hunt based on light conditions and not simply times of day. If lionfish have a difference in hunting activity and a difference in hunting success under different light conditions, it could have implications for their feeding activity across their depth range.

A change in foraging behaviors based on light conditions would not, however, be exclusive to lionfish. Many teleost species have been noted to change foraging behavior or have a difference in hunting success based on a changes in light (Helfman 1986, McMahon and Holanov 1995, Richmond et al. 2004, Rickel and Genin 2005). *Micropterus salmoides* (largemouth bass) was specifically tested for foraging success under different light levels, and were found to have decreased success in starlight, and a drop to zero percent success in darkness (McMahon and Holanov 1995). It was noted in McMahon and Holanov (1995) that behavior during lower light levels and darkness changed, with many more strikes and body movement in the tanks where testing occurred. Richmond et al. (2004) noted that prey detection distances were reduced when *Perca flavescans* (yellow perch) were exposed to low light levels. *Dascyllus marginatus* (Red Sea damselfish), *Neopomacentrus miryae* (Miry's demoiselle), and *Pseudanthias squamipinnis* (lyretail anthias) were noted to have an increase in foraging activity and efficiency during dawn and a decrease during dusk (Rickel and Genin 2005). Helfman (1986) provided a broader overview of fish behavior not centered on a single species, noting that differences in feeding, breeding, aggregation, and rest are often correlated to diel light cycles, either directly or due to changes in predator-prey interactions during these light level changes.

The light environments of mesophotic reefs, which include lower intensity and more restricted wavelengths, may affect the periodicity, duration, and success of lionfish hunting. Lionfish hunting relies at least partially on sight, but it is possible that they could utilize other senses to compensate and hunt in low light conditions such as auditory cues, olfactory cues, and their lateral line similar to other species such as *Perca flavescens* (yellow perch) (Roca et al. 2020), *Poecilia reticulata* (guppy)(Chapman et al. 2010) *Cottus bairdi* (mottled sculpin) (Hoekstra and Janssen 1985), *Silurus glanis* (Wels catfish) (Pohlmann et al. 2004), and *Astyanax mexicanus* (Mexican tetra) (Yoffe et al. 2019). It is unclear how necessary sight is to lionfish hunting, but a heavy reliance on sight would likely affect hunting success in low light. This effect on hunting success could be significant enough to warrant a light-dependent function in lionfish foraging models. Such a modification may be necessary to model lionfish distribution or growth on shallow reefs versus on mesophotic reefs.

Mesophotic reefs should be considered in lionfish management strategies as they are an important habitat for a key portion of the lionfish population (Andradi-Brown et al. 2017, Gress et al. 2017). While spearfishing has been a commonly practiced culling effort, most spear fishers are limited to recreational dive limits of 40 m. This may allow mesophotic reefs to act as refuges for mature and fecund populations of lionfish (Andradi-Brown et al. 2017). The same limits that prevent culling efforts due to depth also make it difficult to observe lionfish populations at these depths, incurring expense and effort, which makes models necessary to plan conservation or monitoring efforts. Models which take into account differences in growth or fecundity based on differing environmental conditions could be used to give good estimates of where the most effective culling should take place without the expense and effort of diving to each individual mesophotic reef.

Several studies in recent years have targeted the idea of a deep reef refugia hypothesis, whereby the mesophotic reefs could serve as refugia for shallow reef species including corals (Bongaerts et al. 2010, Holstein et al. 2015, Semmler et al. 2017) and fishes (Feitoza et al. 2005, Bejarano 2014, Semmler et al. 2017). Lionfish are already known to disrupt mesophotic reef ecology, with the potential to cause a phase shift to algae dominated reefs by way of predation on native species, affecting both fish and coral assemblages (Lesser and Slattery 2011). High abundances of lionfish on mesophotic reefs also skew data on other fishes populations, primarily through predation (Pinheiro et al. 2016). Lionfish have even been found to be preying on previously undescribed or unknown species (Tornabene and Baldwin 2017). Lionfish are capable of feeding and creating issues at mesophotic depths, and could continue to disrupt the species found there, including ones we are not yet aware of. The rate at which they are feeding at mesophotic depths is important for future modeling and conservation efforts of the understudied mesophotic reefs.

To understand how light conditions influence lionfish foraging behavior, we have conducted feeding trials under a range of light conditions. Given examples from the literature of other species experiencing a decrease in feeding success or a change in behavior with decreasing light levels (Helfman 1986, McMahon and Holanov 1995, Richmond et al. 2004, Rickel and Genin 2005), and the presence of a healthy lionfish population in many areas with low light conditions (Lesser and Slattery 2011, Pinheiro et al. 2016, Andradi-Brown et al. 2017, Gress et al. 2017, Tornabene and Baldwin 2017), it is predicted that lionfish will have a lower feeding success when compared to high light areas, but because healthy and fecund mesophotic populations exist, it is expected that they will still have the ability to feed.

1.2. Methods

This study was conducted in the Louisiana State University Marine and Community Ecology Laboratory in January, February, and December of 2020 as well as July and August of 2021. Six opaque white 45 gallon aquariums were wrapped in black plastic that is impermeable to light, so as to create a space in which light could be manipulated, with a small feeding tube placed through the plastic layer. The feeding tube was also covered with a plastic flap of the same material that covered the tanks. Small porous rocks (roughly 12cm by 7cm by 7cm) were placed in the center of the tanks to act as structure and hiding area for lionfish and prey fish. The tanks were part of a recirculating pump system with a 55 gallon sump tank, which included physical and biological filtration, and several unmodified holding tanks. Saltwater was mixed on site for additions and water changes. Temperature and salinity were kept at consistent levels 27-28 °C and 30-32 PSU (Practical Salinity Units) respectively, and were measured daily with a ThermoFisher Scientific Orion 013010MD conductivity meter and an external thermometer. Ammonia, nitrate, and nitrite were tested two to three times weekly in the system utilizing API saltwater test kits. Ammonia and nitrite were kept at undetectable levels, and nitrate was kept below 20ppm for all trials.

Lionfish were collected from Destin, Florida and Summerland Key, Florida on both natural and artificial reefs via scuba and hand nets. Depths of collection ranged from 12 m to 24 m, and a commercially available swim bladder venting tool was used as needed to vent gas that had expanded within the swim bladder. Lionfish were transported by coolers equipped with bubblers within vehicles. After transportation back to the Marine and Community Ecology Laboratory, lionfish were allowed to acclimate in the system for a minimum of one week, and in

the trial tanks for an additional week. For two days before the trial, lionfish were fed frozen krill and silversides until they no longer willingly ate twice per day.

All prey fishes utilized were reef dwelling damselfishes, and were randomized within trials. Species included *Dascyllus trimaculatus*, *D. aruanus*, *D. reticulatus*, *D. melanurus*, *Chrysiptera cyanea*, *C. parasema*, *C. hemicyanea*, *Pomacentrus smithi*, *P. moluccensis*, and *P. lepidogenys*. Prey fishes were all obtained from pet stores located in Baton Rouge, Louisiana. Prey fishes were maintained at the LSU Marine Community Ecology Laboratory in 45 gallon aquariums with temperature and salinity 27-28 °C and 30-32 psu, respectively. Prey fishes were fed frozen mysis shrimp daily.

Trial tanks were each equipped with 5MP, 1080p, IR turret cameras (Supercircuits item H5T682KT), suspended above and in the center of the tank. Cameras were used to count remaining prey fish and were not used to determine lionfish behavior. Each tank was also equipped on one side with a small, magnetically mounted white LED light modified to be externally wired for constant power output. The lights were set up with four different treatments; one with unfiltered light, two which had different light filters fitted, and one light which was kept off. The light filters utilized were a Deep Blue 47 Kodak Wratten Color Filter, which has a peak of approximately 50% transmission at 440 nm, and a FSQ-BG18 Colored Glass Bandpass Filter which peaks at roughly 70% transmission at 493 nm. This resulted in four light conditions, hereafter referred to as “treatments”: “light” (unfiltered LED lighting), “light blue” (70% transmission at 493 nm), “dark blue” (50% transmission at 440 nm), and “dark” (no lighting). Lights were set on an external timer which turned them on at 7:00 am and off at 9:00 pm daily. All external cords were sealed with black plastic and electrical tape to allow no ambient light entry.

Each trial consisted of all four light treatments running for 48-72 hours. A baseline of 5 prey fishes were added to each tank for the beginning of each trial. Prey fishes were replenished three times daily up to the 5 fish baseline. All prey fish weights (g) were measured before addition to the tanks, and any remaining at the end of each trial were removed and remeasured. Lionfish weights (g) and total length (cm) were recorded at the end of each trial. Lionfish were weighed and measured directly after the end of each trial by placing them, alive, in a white plastic box with tall sides on a scale tared for its weight and covered with a semi-transparent plexiglass material to prevent envenomation of the trial conductors. Prey fishes were patted gently with dry paper towels before being measured on the scale, to reduce the potential weight difference of water droplets.

The weight of prey fishes consumed in each trial (g) was calculated by subtracting the weight of the prey fishes remaining in the tank from the weight of the fishes added to the tank throughout the trial. Weight consumed was divided by the length of the trial in days to calculate prey weight consumed per day. Weight consumed per day was analyzed using ANCOVA, with the weight of the lionfish used in the trial included in the model as a covariate, due to consumption being positively correlated with weight, and light treatment included as a main effect. Visual inspection of the quantile-quantile plot and distribution of residuals indicated that the data met the assumptions of the ANCOVA model. A Type II ANOVA was used to analyze the ANCOVA model, because the model did not include any interaction effects. For main effects significant at $\alpha = 0.05$, a Tukey's multiple comparison test was performed to examine differences in means. Two-treatment comparisons were considered significant at $\alpha = 0.10$.

The wavelength and intensity of the light passing through each filter was matched to expected light conditions at various depths in the ocean. Data characterizing the relative intensity

of sunlight at the ocean surface were extracted from Figure 1 in Muaddi and Jamal (1991) using WebPlotDigitizer (<https://automeris.io/WebPlotDigitizer>). Light attenuation coefficients for 350 to 500 nm wavelengths at intervals of 5 nm were extracted from Figure 6 in Lee et al. (2015) using WebPlotDigitizer. Relative intensity of light at depth was calculated using the equation for light attenuation:

$$I_z = I_0 e^{-kz} \quad \text{Equation 1}$$

Where I_0 = irradiance at the surface, I_z = irradiance at depth z , and k = the attenuation coefficient (wavelength-specific). The depth that exhibited roughly 70% transmission at 493 nm was matched to the “light blue” treatment, and the depth that exhibited 50% transmission at 440 nm was matched to the “dark blue” treatment. The dark blue filter was subject to pigment degradation throughout the experiment, which likely shifted the spectrum slightly. Results that include analysis of depths calculated in this manner are treated with caution due to the degradation of the dark blue filter. Sensitivity of the results to the choice of depth for the “dark blue” treatment was examined for models that included depth as a variable.

To examine the relationship between lionfish consumption and depth, the weight of prey fishes consumed by each lionfish used in the experiment was divided by the weight of the lionfish (g) and the length of the trial (days) to calculate prey weight consumed gram^{-1} of lionfish day^{-1} . Three functions were fit to the data to describe the relationship between prey consumed ($\text{g g}^{-1} \text{d}^{-1}$) and depth (m): a linear function, a polynomial function, and an exponential function. The linear function took the form:

$$y(z) \sim y_0 + az \quad \text{Equation 2}$$

where $y(z)$ = the consumption at a given depth z , y_0 = the intercept, and a = the slope of the line.

The polynomial function took the form:

$$y(z) \sim y_0 + az + bz^2 \quad \text{Equation 3}$$

where $y(z)$ = the consumption at a given depth z ; y_0 = the intercept; and a and b are parameters which determine the shape of the polynomial function. The exponential decay function took the form:

$$y(z) \sim y_f + (y_0 - y_f)e^{-e^{(\log \alpha)z}} \quad \text{Equation 4}$$

where $y(z)$ = the consumption at a given depth z , y_0 = the intercept, y_f is the asymptote, and α controls the rate of decay. This model approximates the fit of an exponential decay function (Eq. 5), but is much more likely to converge in R.

$$y(z) \sim y_f + (y_0 - y_f)e^{-\alpha z} \quad \text{Equation 5}$$

All models were fit using nonlinear least squares in R. The models were compared using Akaike's Information Criterion (AIC), and the best model as identified by AIC was chosen to represent the relationship between lionfish consumption rates and depth.

1.3. Results

The lionfish weights varied from 17.4 g to 254.4 g, with an average of 140.1 g, and their total lengths ranged from 12.5 cm to 26.0 cm. Sizes of damselfishes ranged from 0.4 g to 2.6 g. The overall average consumption was 0.034 g g⁻¹ d⁻¹. The overall maximum consumption was 0.069 g g⁻¹ d⁻¹, and the overall minimum was 0.013 g g⁻¹ d⁻¹. The average consumption for the “light” trials was 0.049 g g⁻¹ d⁻¹, 0.032 g g⁻¹ d⁻¹ for “light blue”, 0.030 g g⁻¹ d⁻¹ for “dark blue”, and the average consumption for the “dark” trials was 0.026 g g⁻¹ d⁻¹.

The ANCOVA analysis identified that light condition significantly affected lionfish consumption rates (Table 1; Figure 1). Follow up post hoc tests indicated that there was some evidence that lionfish consumption rates were greater in the “light” treatment than in the “light blue” or “dark” treatments (significant at $\alpha = 0.10$; Figure 1). Lionfish consumption rates in the “dark blue” treatment was not significantly different from any other light treatments (versus “light”: $t = 1.42$, $p = 0.51$; versus “light blue”: $t = -1.11$, $p = 0.69$; versus “dark”: $t = 0.99$, $p = 0.76$). Consumption rates in the “light blue”, “dark blue”, and “dark” treatments were all very similar and could not be distinguished statistically (Figure 1).

Table 1. ANCOVA results.

	Df	Sum Sq	F-value	Pr(>F)
<i>Lionfish Weight</i>	1	31.89	14.54	0.002
<i>Light Treatment</i>	3	21.63	3.29	0.05
<i>Residuals</i>	15	32.90		

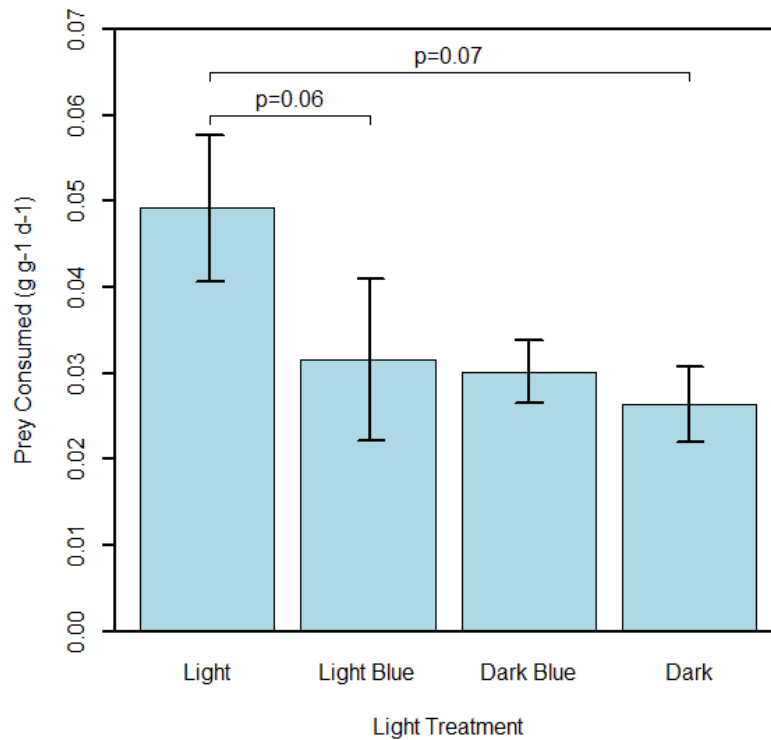


Figure 1. Light treatment vs. Prey consumed ($\text{g g}^{-1} \text{d}^{-1}$), error bars are standard error, sample size: “Light”: 5, “Light Blue”: 5, “Dark Blue”: 4, “Dark”: 6. “Light” treatment was significantly different from the other treatments.

The analysis of light attenuation with depth (Figure 2) resulted in the association between light treatments and depths as follows: “light” was 0 m depth, “light blue” was 35 m depth, “dark blue” was 80 m depth, and “dark” was 700 m depth. At 35 m, relative intensity exhibited two peaks: one at 70% for wavelength ~ 440 nm, and one at 68% for wavelength ~ 465 nm (Figure 2). At 80 m, relative intensity peaked at $\sim 50\%$ for wavelength ~ 435 nm (Figure 2). Nearly all wavelengths were fully absorbed at 700 m depth, with peak relative intensity $< 1\%$, so this was considered an appropriate depth to associate with the “dark” treatment (Figure 2). The LED light

used was 6500K, a hue that approximated daylight, so the “light” treatment was associated with surface daylight conditions.

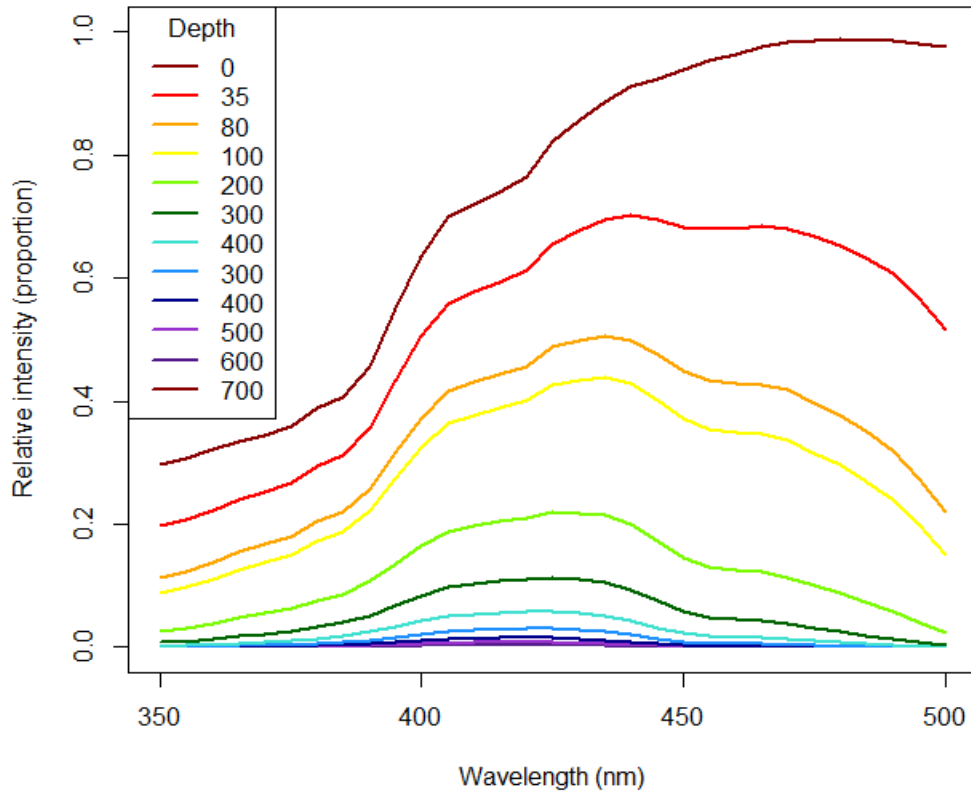


Figure 2. Wavelength (nm) vs. Relative Intensity (proportion). Depths 0-700 plotted, showing the modeled light attenuation with depth.

Model comparison using AIC indicated that the exponential decay function was the best model of the three candidate models (linear, AIC = -103.5; polynomial, AIC = -104.7; and exponential, AIC = -105.8). The exponential decay of lionfish consumption rate as a function of depth took the form:

$$y(z) \sim 0.027 + 0.022e^{-0.044z} \quad \text{Equation 6}$$

The asymptote of the model was $0.027 \text{ g g}^{-1} \text{ d}^{-1}$, and this asymptote was reached at $\sim 200 \text{ m}$ depth (Figure 3).

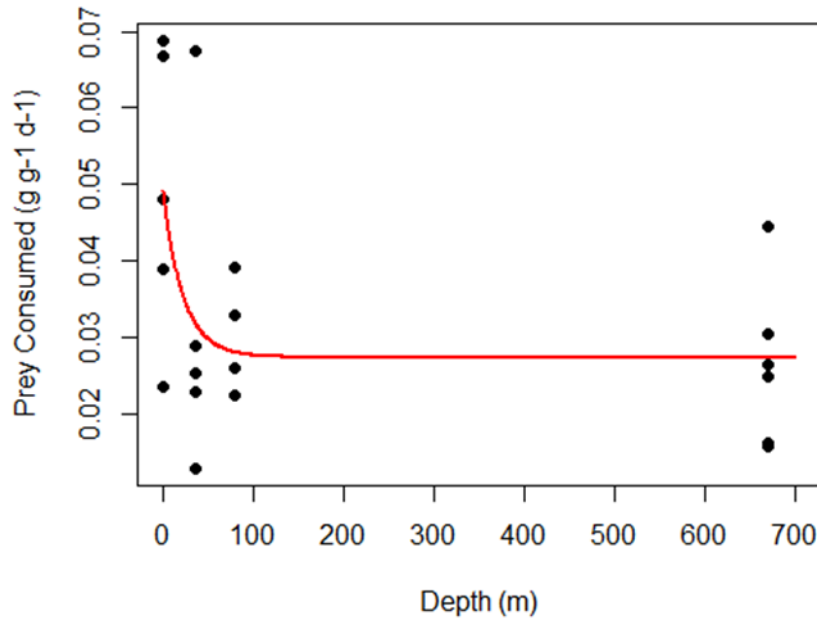


Figure 3. Depth (m) vs. Prey Consumption ($\text{g g}^{-1} \text{ d}^{-1}$), Exponential decay from Equation 6 plotted in red, asymptote at $0.027 \text{ g g}^{-1} \text{ d}^{-1}$, showing that lionfish feeding slows with depth but does not stop.

The degradation of the dark blue light filter decreased the amount of blue pigment on the plastic backing, so sensitivity analysis was conducted by decreasing the depth associated with the “dark blue” treatment from 80 to 35 m. The dark blue filter appeared visibly darker than the light blue filter, even when degraded, so this range most likely encompassed the wavelength of the degraded dark blue filter. The asymptote y_f increased slightly as depth increased (Figure 4). The intercept y_0 varied but remained between 0.0491 and 0.0492 (Figure 4). The rate of initial decline α also varied but remained between -3.07 and -3.21 (Figure 4).

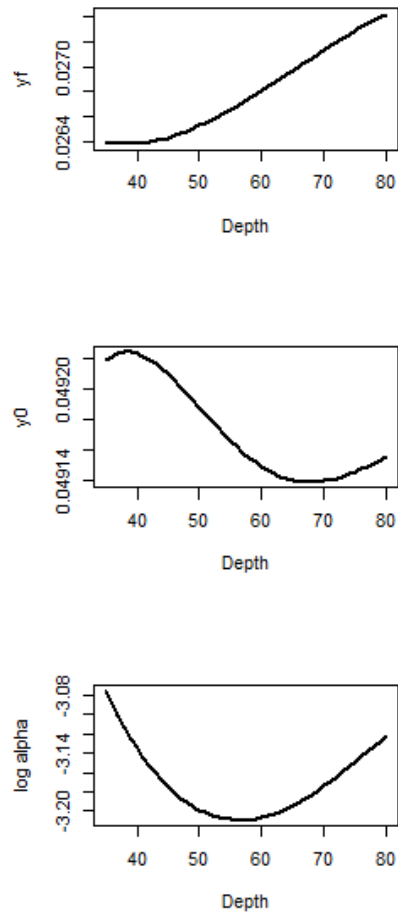


Figure 4. Sensitivity analysis; Depth vs. Asymptote (y_f), Depth vs. Y intercept (y_0), and Depth vs $\log \alpha$. When considering the Y axis, little change occurred.

1.4. Discussion

This study suggests that lionfish vary their feeding rates based on light conditions. There were significant differences between the feeding rates of “light” and “light blue”, as well as “light” and “dark”, however no significant differences were found between the “dark blue” and other treatments. The average rates for “light blue”, “dark blue”, and “dark” were all similar to each other, but there was high individual variability in feeding, likely due to differences in body weight which was accounted for in analysis, which caused wide ranges for each treatment.

The data suggesting that light conditions do negatively affect feeding rates was unsurprising, given the partial reliance on sight and similar effects in other species. On the other hand, the lack of difference between the “light blue”, “dark blue” and “dark” light treatments was surprising and has some interesting potential implications. This result may indicate that lionfish feed at similar rates in diverse reduced light conditions, specifically light typical of ~200 m according to our model using wavelength-specific attenuation coefficients typical of the open ocean (Lee et al. 2015). It is necessary to consider that the attenuation values used in this study were taken from clear, oligotrophic water far from a coast. In coastal areas where lionfish are found and where turbidity, nutrients, and local weather increase light absorption with depth as compared to the open ocean (Anthony et al. 2004, Davies-Colley and Smith 2001), it is expected that similar light conditions would be reached at much shallower depths. As an example, Runcie et al. (2008) measured values of less than 1% surface irradiance near 100 meters, and less than 0.1% roughly near 125 meters. These depths would be considered “dark” within the bounds of the model in this study, and both depths are well within the mesophotic distribution of lionfish in their invasive range. When this idea is combined with data from this study it suggests that a

decrease in feeding rates may occur at much shallower depths than expected, and that feeding rates may be consistent at depths beyond that.

The degradation in the “dark blue” filter did not appear to impact the results of this study. Even though there was slight degradation of the pigments on “dark blue” treatment filter, it still appeared visually darker than “light blue” filter, meaning the actual depth that corresponded to the degraded “dark blue” filter was somewhere within the bounds of the sensitivity analysis. The sensitivity analysis indicated that changing the depth associated with the “dark blue” filter resulted in very slight differences for the parameters controlling the asymptote, intercept, and rate of decline of the exponential decay function. Thus, we are confident in the results of this study, and it is clear the largest change in lionfish consumption occurs with a small reduction in light intensity and/or shift in wavelengths, such as the difference between daylight and light conditions typical of ~35 m depth.

The consumption rates measured during the course of this study were within or close to the ranges of other estimates of lionfish consumption from the literature. Côté and Maljković (2010) observed predation rates of lionfish on Bahamian coral reefs, and their work suggests an average feeding rate of $0.038 \text{ g g}^{-1} \text{ d}^{-1}$. Green et al. (2011) observed activity and feeding behaviors of lionfish on similar Bahamian coral reefs and calculated an average consumption of $0.089 \text{ g g}^{-1} \text{ d}^{-1}$. Fishelson (1997) observed feeding behaviors of lionfish in the Gulf of Aqaba, which is part of their native range, and the data suggest a consumption rate for *P. volitans* of $0.024 \text{ g g}^{-1} \text{ d}^{-1}$. Cerino et al. (2013) assessed many aspects of lionfish physiology and biology, and tested feeding rates in many temperature conditions. From the data presented by Cerino et al. (2013), the mean consumption rate for 27°C was estimated to be $\sim 0.060 \text{ g g}^{-1} \text{ d}^{-1}$. The mean

consumption rate calculated for all treatments within this study, $0.034 \text{ g g}^{-1} \text{ d}^{-1}$, was within the range of previous literature estimates.

The variability in the estimates of lionfish consumption rate is likely due to the wide variety of methods used to quantify lionfish feeding behavior. Both Côté and Maljković (2010) and Green et al. (2011) used scuba divers to follow lionfish and estimate consumption rates on the reefs where the study took place. This introduces error as divers are not typically good estimators of size and distance (Harvey et al. 2002, Harvey et al. 2004). Attempts by divers to classify accurate size of small prey fishes which move quickly, especially while being actively hunted, could skew data significantly compared to fish size and weight measured in a laboratory setting. Using divers to estimate lionfish sizes also introduced sources of error, as lionfish feed differently based on their size (Cerino et al. 2013). Under or over estimating both prey and lionfish size could explain some of the differences between feeding rate values found.

Fish behavior at different densities may also play a role in consumption rate discrepancies. Lionfish have been known to forage differently (Benkwitt 2016) and have different growth rates (Benkwitt 2013, Dahl et al. 2019) at different densities, and both of these are tied to consumption rates. This could be due to intraspecific competition or cooperation during hunting (Kendall 1990). It is likely that differing densities on reefs, especially between the native and invaded range (Green and Côté 2009, Dahl and Patterson 2014, McTee and Grubitch 2014, Trégarot et al. 2015), and solitary conditions in laboratory trials affect the calculation of lionfish consumption rates.

In addition, previous studies observed lionfish under lighted laboratory conditions or non-mesophotic depths in the wild, meaning that several light conditions and wavelengths represented in this study were not represented in the previous studies. These differences would

likely result in our study underestimating the mean consumption rate of lionfish as compared to estimates from other studies. As a crepuscular feeder, lionfish activity level is known to vary with light availability. Côté and Maljković (2010) noted that lionfish were more active during days that were overcast in their study. Though previously untested and not directly a part of this study, there is the potential for lionfish at consistently lower light conditions, such as on mesophotic reefs, to be hunting for longer periods each day or even all day as opposed to distinct peaks during crepuscular times as they do on shallow reefs.

Night represents a large portion of the diel cycle, but lionfish feeding during the night is poorly understood, with several previous studies excluding it from their observations (Morris and Akins 2009, Côté and Maljković 2010, Green et al. 2011). Both Côté and Maljković (2010) and Green et al. (2011) did make observations during crepuscular periods and during the day, but not at night. Green et al. (2011) made the assumption that no predation was possible by lionfish at night, and thus did not include it in their model. The data found in this study clearly shows that feeding in complete darkness is not only possible, but that the rate of feeding is also similar to depths with low light conditions during the day. While it is possible or even likely that lionfish hunt preferentially during crepuscular periods, predation at night or depths with no light should not be discounted as zero. The reason for continued predation in low light and darkness is, however, unclear. No literature data was found on the specifics of the visual and overall sensory system usage in lionfish. Their potential usage of UV light, auditory cues, lateral line, or olfactory systems remain unclear. The rates found in this study should be considered for future bioenergetics models instead of excluding night or depths with no light, however, other physical and biological factors associated with depth, such as temperature, pressure, currents, salinity, fish

and invertebrate assemblage makeup, and compensation with other sensory systems should also be studied and considered when creating these models.

It is also worth noting that this study had high variability in a few of the results, which could be due to the individuality in hunting behaviors of specific fish, or due to body weights given the relatively small number of subjects used compared to their density on the reefs. In addition, all of the lionfish and likely the damselfishes were collected from reefs that were shallower than mesophotic, and there could be differences in sensory adaptations affecting hunting and predator avoidance between the shallow and mesophotic reefs. It is likely that studying much larger populations would reduce variability, but the low number of animal subjects used in this experiment was out of necessity due to several logistical issues obtaining and maintaining lionfish in captivity, including an unidentified virus present in several populations (Harris et al. 2020).

The scope of this laboratory study did not include many other potential factors which should each be tested individually to determine their effect on hunting success. The same light intensity output was used for all filters and full light treatments, but further studies could change this intensity, either in combination with light wavelengths to match mesophotic reefs more closely, or light intensity on its own to determine what effect this specific factor has. Temperature was also kept reasonably constant, but seasonal and permanent thermoclines are present in the wild. Turbidity and salinity can vary as well, especially near shorelines, but neither were changed for this study.

Several previous studies (Andradi-Brown et al. 2016, Andradi-Brown et al. 2017, Gress et al. 2017) provide evidence that lionfish are extending their reach to deeper reefs than we can effectively cull by regular means such as spearfishing. Not only are they at these depths, but they

are thriving with many large, fecund adult specimens with full stomachs being found in high densities. This is indicative in some way of high hunting success, though what drives this success is unclear. Lionfish are primarily crepuscular hunters, and they are able to hunt in low light conditions, which may add to success on deeper reefs (Cure et al. 2012); however, this may not be the only reason. Prey naiveté to a novel predator may play a role in hunting success in lionfish, with some studies reporting little to no difference in prey avoidance behaviors when compared to native predators (Black et al. 2014), but with many more studies reporting that avoidance behaviors in prey species were less pronounced or absent when exposed to the novel predator (lionfish) as compared to exposure to native predator species (Marsh-Hunkin et al. 2013, Anton et al. 2016, Agostino et al. 2020). If some native prey species are not avoiding lionfish at the same rate as native predators, it may give lionfish an advantage at depths with low light availability, allowing them to survive and grow even under conditions to which they are not adapted to thrive.

With many factors potentially affecting the hunting success of lionfish, especially as they extend to new depths and habitats, it is important to discern which specific factors have an effect on their success. This study attempted to single out a specific potential factor, which was varying relative intensity and wavelength of light, in an attempt to mimic mesophotic light wavelengths. Lionfish consumption rates dropped off with a small reduction in light intensity and dominant wavelength typical of ~35 m depth, and consumption rates remained fairly consistent with further light reduction. Even in darkness, lionfish consumption rates did not reach zero. This suggests that when future modeling and conservation efforts are undertaken, values for feeding rates should be similar for a large range of the mesophotic populations of lionfish, even populations residing on rocky features in complete darkness.

Chapter 2. Unbaited Stereoscopic Video Traps

2.1. Introduction

Understanding fish behavior is important to understanding how fish interact with their environment, however, it can be difficult to study, as it has many facets, and there are many approaches to do so (Rowland 1999). Rutecki et al. (1983) notes that before the behavioral patterns of a species are known, all movements, patterns and changes must be studied since they could be useful. These could include fin movements, gill movements or flares, changes in body position or angles, and even color changes. Rowland (1999) suggests similar cues for analyzing fish behavior, emphasizing shape, size, posture, specific morphological structures, marking patterns, and movement. He also notes that these categories can overlap, including with multiple cues being used, with nonvisual cues, as well as with the perception of the analyst.

Analyzing behavioral cues is necessary because of the impacts that fish behavior can have on conspecifics as well as the wider ecosystem. Lionfish show density dependent growth, which is not a behavior itself, but which indicates a change in behaviors (Benkwitt 2013), foraging behavior (Benkwitt 2016), and movement (Tamburello and Côté 2015), and changes in these behaviors can affect other lionfish, and could affect native fish densities. Bassar et al. (2012) found that *Poecilia reticulata* (guppy) was able to decrease local algal and invertebrate populations when their foraging behavior increased after a change in density. Gil and Hein (2017) found that when they observed all species on a patch of reef there were density dependent bursts of feeding and foraging where fish changed their behavior and feeding rates based on the density of other species in the area. Knowing specific behavioral cues in fish can help us quantify behaviors that affect intraspecies and interspecies interactions.

Lionfish (*Pterois volitans* and *P. miles*) exist in much higher densities in their invaded range as compared to their native range (Green and Côté 2009, Dahl and Patterson 2014, McTee and Grubitch 2014, Trégarot et al. 2015). Dense aggregations of lionfish results in density-dependent behaviors indicative of intraspecific competition, which result in extra time spent foraging (Cure et al. 2012, Benkwitt 2013, Benkwitt 2016). In contrast, there is some evidence that lionfish may work together to hunt in groups, increasing foraging efficiency by working together to attack a school of prey fishes (Kendall et al. 1990, Lönnstedt et al. 2014, Rizzari and Lönnstedt 2014). Understanding lionfish behavior could lead to a greater understanding of how these behaviors affect the wider ecosystem, such as native fishes and coral populations (Albins and Hixon 2008, Lesser and Slattery 2011, Green 2011, Benkwitt 2014) In general, social behavior in lionfish is not very well studied, but given that lionfish exhibit density dependent behaviors as well as an increased density in their invaded range it is even more important to understand specifically why certain behaviors are displayed.

Swimming speed often differs with behavior, such as foraging, mating, or fighting (Price 1989, Brownscombe et al. 2014, Marras et al. 2015). Swimming speed is a key parameter in foraging models because it can determine how much energy is expended, as well as how fish assess and interact with their environment to gain food for energy (Boisclair and Sirois 1993, Brownscombe et al. 2014). Energy gains and expenditures are the basis of bioenergetics models and since swimming speed is involved in both, it can be an important behavioral factor to measure (Hewett and Johnson 1992, Boisclair and Sirois 1993, Brownscombe et al. 2014).

Swimming speed is difficult to measure because of the need for precise measurements of both distance and duration of the behavior. While diver surveys are useful, and perhaps even necessary, they potentially disturb the behavior of the organism being analyzed, they are very

limited in their time and depth, and they lack accuracy and precision (Rutecki et al. 1983, Harvey et al. 2002, Cappo et al. 2003, Harvey et al. 2004, Mills et al. 2005). Remote camera systems have little to no disturbance on fish behavior and can be deployed for long durations and without depth limitations (Rutecki et al. 1983, Harvey et al. 2002, Cappo et al. 2003, Harvey et al. 2004, Mills et al. 2005). In order to assess visual cues associated with fish behavior, remote camera deployments are undoubtedly a necessity. Using photogrammetry by way of stereo-camera setups is ideal for analyzing the minutiae of lionfish behaviors because it can include the benefits of remote video traps coupled with precise measurements, both of movement in 3-dimensional space as well as time.

Photogrammetry, or the measuring of objects from photos, is not a novel concept, and various forms of it have been in use for many years (Doyle 1963). Protocols for photogrammetry vary greatly depending on the goal of the project and the methods used to obtain measurements. Basic photogrammetry with a single camera will typically use multiple photos aggregated at a later time to determine measurements or create models (Linder 2009). This method is useful for stationary objects, but it does not generally work for moving objects, and especially not for objects with quick movement such as fish.

In contrast to single camera protocols, in a stereo-camera or stereoscopic system two cameras are attached to a bar with a known separation distance and angle of convergence (Letessier et al. 2013), which allows measurements in three dimensions with respect to the cameras with each pair of photographs taken. A stereo-video system allows measurements to be taken with respect to time as well as space. This allows the in-situ measurement of attributes that previously were unable to be measured with acceptable accuracy and precision. As an example, previously measuring lengths of organisms in situ would require them passing in front of or near

to some preset scale, be it two lasers such as on ROVs, a measuring stick, or an object of known length. How close the organism passed to the preset scale, in which direction they passed it, and at which angle all affect the accuracy of the measurements. Calibrated stereo-video systems, on the other hand, can be used to measure the lengths of the same organisms but without the constraints (Harvey and Shortis 1995, Harvey et al. 2002, Harvey et al. 2004). A fish can be within a large range of distance of the camera system, and at nearly any angle or orientation, and can still be accurately measured.

Stereo-video systems are not perfect, however. Drawbacks include increased cost for systems and software, as well as increased complexity with respect to calibration and measurement as opposed to single camera setups. Slight errors can also occur in stereo-video measurement, although they are significantly lower than error from diver measurements (Harvey et al. 2002, Harvey et al. 2004). As with any measurement device errors occur and the functionality of these systems is not perfect or endless in scope, but the use of stereo-camera systems allows for far more accurate measurements than most alternatives.

Lionfish behaviors have occasionally been considered in previous studies (Green et al. 2011, Cure et al. 2012, Benkwitt 2016), but typically with a focus on the amount and type different behaviors, with little regard to the specifics of the behavior itself. In addition, studies on large scale movements between reefs showing site fidelity have been conducted (Bacheler et al. 2015, Tamburello and Côté 2015, McCallister et al. 2018), but to a degree there exists a gap with respect to how lionfish movement along a reef changes with different specific behaviors such as resting or hunting. Understanding more specific, fine scale behaviors and actions could be an important factor to accurately model their spread and behavior in an effort to make appropriate management decisions to mitigate damage.

This study used stereo-video camera systems to examine the behavior of lionfish cohabiting shallow patch reefs in the Florida Keys, USA. The primary objectives of this study were to identify incidences of density-dependent behavior from the camera footage, and to quantify swimming speeds associated with different behaviors. We hypothesized that lionfish cohabiting a reef would exhibit density-dependent behaviors such as aggression and cooperation in foraging.

2.2. Methods

Unbaited video traps with stereoscopic camera systems (hereafter, camera systems) were used to measure lionfish movement speed and to examine lionfish behavior on shallow patch reefs in the Florida Keys, USA. Patch reefs were filmed before dusk for a period of roughly 3 to 4 hours (limited by the battery capacity of the cameras) and recovered the next morning. Three camera systems with two cameras each were used to collect data each deployment, and several deployments were made in different areas to capture footage of different fish. Five days of footage were collected from June 12th to June 17th.

2.2.1. Site selection

Camera systems were deployed near several patch reefs < 20 m depth in the Florida Keys off Summerland Key, and near both Looe Key National Marine Sanctuary and the American Shoal Lighthouse. Each patch filmed had 2-6 lionfish in an area less than 100 m². Although the camera systems were placed at several reefs, the primary patch reef used was at 24.537 °N, - 81.435 °W, at 15-16 m depth, with mean water temperature 28.5 °C and no discernable current (Figure 1). Visibility generally ranged between 20 and 30 meters between days, but never less than 15 meters, even after storms. The patch was roughly 15 m across, 5 m wide, and 2 m in height, with good vertical relief and many overhangs. The reef was surrounded with sand and patchy rubble, and the nearest patch reefs were roughly 30 m away in any direction. Lionfish prey such as small crustaceans, damselfishes, and wrasse were abundant. This particular patch was chosen because it represented good average lionfish habitat and because the resident lionfish tended to stay in a localized area, only occasionally willing to go to nearby reefs. This made it ideal to get as much video footage as possible of the lionfish.

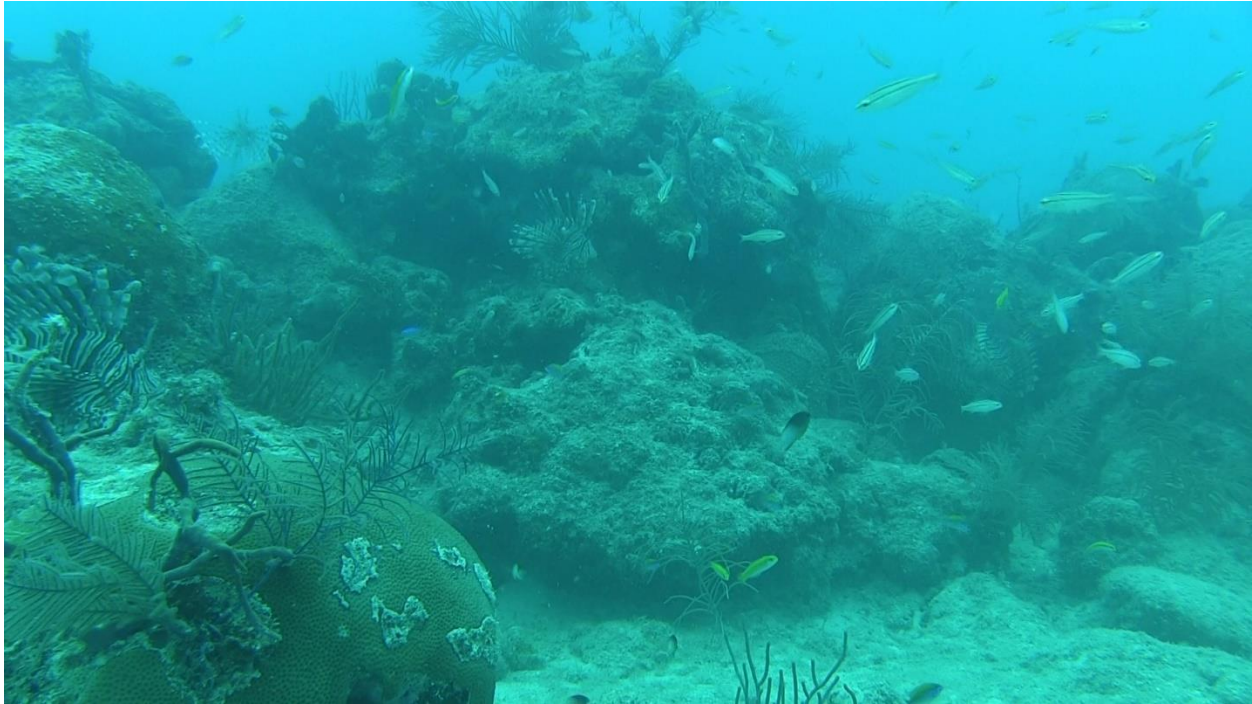


Figure 1. Florida Keys patch reef, 24.537 °N, -81.435 °W, at 15-16 m depth.

2.2.2. Camera system

The camera system used in this project was custom built by SeaGIS (<http://www.seagis.com.au/>). The camera mount was an aluminum bar 75cm in length, and 10cm wide, with angled housings attached on each end that pointed the cameras toward each other so that their field of view overlapped (Figure 2). When deployed in the water, the camera system housing was bolted to a frame made from steel, with a width of 40.5cm, a length of 88cm, and a height of 53cm. The purpose of the frame was to weight the camera system to keep it on the bottom, and to keep the cameras stable and off the sand.



Figure 2. SeaGIS stereo camera housing.

The cameras used were Canon HFG10 models, 1080p resolution, with settings mode set to M and FXP recording. A 60i frame rate was used leading to a working frame rate of 29.97 frames per second. Video files were saved to SanDisk Ultra SDXC 30MB/s 64gb external SD cards. Canon brand lithium-ion batteries were used to extend recording times.

For calibration purposes six total video cameras were enclosed in paired underwater housings and brought to a pool with the SeaGis 3D calibration cube, where initial footage was taken to be processed in the SeaGis CAL™ (<http://www.seagis.com.au/>) software. In this software, bundle adjustments were made for calibration, and camera files were obtained for each individual camera system. Final video footage from the field was combined with the calibrated camera files, and the SeaGis CAL™ software EventMeasure™ (<http://www.seagis.com.au/>) was used to synchronize the two video files and allow measurement in 3D (Letessier et al. 2013).

2.2.3. Behavior characterization

A wide variety of lionfish behaviors were observed in the recordings. While behaviors such as aggression were observed, noted, and will be described in the results and discussion, they were not quantifiable, or were not common enough to be replicated for quantification.

Swimming speeds were classified under three different observed behaviors: relaxed swimming, traverse swimming, and striking at prey. The differences between these behaviors were primarily distinguished based on body and fin positioning, as well as the apparent intent of the motion if any was evident. Durations of measurement varied, but were typically less than one minute long. Durations were judged by when fin positioning movement changed fully to the described behaviors to when body momentum ceased and fin positioning changed again. Fin positioning included primarily dorsal spines and pectoral fins, which are further broken down into dorsal, medial, and ventral segments.

Relaxed swimming was characterized by a very slack, low effort, forward swim, with a neutral positioning of all fins (Figure 3). Dorsal spines were near fully or fully erect, and pectoral fins lay primarily to the side of the body, neither flared nor raised. The dorsal section of the pectoral fin was typically near the midpoint of the fish both horizontally and vertically, though a small flare both out and up was usually present. The medial portion of the fin was typically parallel or close to parallel to the main body of the fish, and the ventral portion came close to mirroring the dorsal portion. This was the position that had the widest variation, though the low frequency at which the caudal fin moved and the lack of any noticeable object or prey the body was pointing towards also helped to classify a swim as relaxed.



Figure 3. Relaxed forward swimming; all fins in a neutral position, no excessive flaring

Traverse swimming was distinct from relaxed swimming in that the dorsal spines as well as all three parts of the pectoral fins would completely fold back against the body, and the



Figure 4. Traverse swimming; Dorsal and pectoral fins both folded into body, rapid caudal fin movement follows moving the fish forward

frequency of caudal fin movement increased rapidly, propelling the fish forward (Figure 4). This type of swimming appeared to be intentioned to traverse small distances very quickly though the reason was not always clear, and not always captured in the footage. The body position for traverse swimming typically included a sudden pivot away from where the lion was currently starting, and the end point if visible was either an object or another fish (though not always a prey-sized organism). While rare, it also seemed to be used to escape much larger organisms, potentially those seen as a threat to the lionfish, such as approaching human divers.

Striking at prey was always preceded by a hunting behavior which included two types of fin and body positioning. The body position for of a lionfish striking at prey was almost always angled directly at a prey organism, leading often to a perpendicular body position with reference to the substrate, rock, or coral head. The dorsal spines were erect for lionfish striking at prey. The fin position altered between 1) a full flare of the pectoral fin vertically perpendicular to the body, where most or all of the three portions of the pectoral fin were extended in a vertical semi-circle on either side of the body (Figure 5); and 2) a horizontal flare of the dorsal portion of the pectoral, with the medial portion tucked against the body, and the ventral either pointing down or also tucked against the body (Figure 5). The prey strike itself consisted of a rapid lunge towards the prey with a swift beating of the caudal fin, while dorsal spines and pectoral fins were folded back against the body, similar to the traverse swim. Key differences between this behavior and the traversal swim were the hunting behaviors described above preceding the strike itself, the presence of an apparent prey item or items, and a much shorter distance and duration of the swim (often only a few frames). These differences can also be used to differentiate prey strikes from other similar short and quick movements, such as after yawning and during aggression.



Figure 5. Horizontal and vertical fin flares preceding a forward strike with body positioning towards prey school and substrate

2.2.4. Swimming speed

Swimming speeds were calculated using measurements from previously calibrated video footage using the program EventMeasure™. Some of the footage was internally edited in EventMeasure™ to change the brightness and contrast, making measurements easier to obtain. These were temporary edits, and did not affect the measurements themselves, as timing and scale of the footage remained unchanged. The benefit of photogrammetric measurements is that movement in any direction can be measured. However, when attempting to calculate a speed at which direct swimming movements occur, large amounts of erratic lateral movement, or winding paths will not give an accurate idea of the speed of the movement in one direction, regardless of

which direction that may be. Thus, to reduce the error from winding or non-direct paths, an attempt was made to primarily measure straight line movements.

When a lionfish was observed to swim unobstructed across the camera field of view, a segment in which the lionfish was traveling in a straight line was used to calculate swimming speed. The location of the lionfish at the beginning and end of the swim was determined as the midpoint of the lionfish, as determined by a direct line from the center of the eye to the middle of the last stripe of each fish. This measurement was used because the caudal fin of lionfish is both translucent, making it hard to capture in some video, as well as easily damaged, meaning that not all lionfish have equally proportioned caudal fins relative to their body size. Measuring from the eye to the middle of the final stripe allowed easier and more consistent measurements than attempting total length. The midpoint of this measurement was used to account for small variations in body angle and movement of extremities. Measuring solely the eye, tip of mouth, or other landmarks on the fish would give less accurate measurements of whole-body movement. For prey strikes, although the mouth does move independently of the body as the jaw protrudes, the movement of the mouth was not measured independently due to limitations of the camera system, and only the whole-body movement was measured. Rarely, the eye to final stripe measurement was not possible, in which case a measurement from the eye to the center of an easily visible stripe was taken, and the same measurement was used in each frame.

Measurements taken in the software were exported to excel, and the 3D distance formula combined with the division of time was used to calculate the swimming speed.

$$\textit{Swimming Speed} = \frac{\sqrt{(X_2-X_1)^2+(Y_2-Y_1)^2+(Z_2-Z_1)^2}}{(T_2-T_1)} \quad \text{Equation 1}$$

Where X_1 , Y_1 , and Z_1 were the initial midpoint position of the lionfish in millimeters, and X_2 , Y_2 , and Z_2 were the final midpoint positions of the lionfish in millimeters, T_1 was the time when the fish was present at the initial point in seconds, and T_2 was the time when the fish was at the final point in seconds. The top portion of this equation yielded the distance in millimeters traveled by the lionfish, and the bottom half yielded the amount of time in seconds the lionfish took to travel the distance, giving in total the speed at which it was swimming in mm s^{-1} .

2.2.5. Analysis

A Shapiro test and a Levene test were run to assess the ANOVA assumptions of normality and homogeneity of variance. A square root transformation was applied to swimming speeds to meet the assumption of homogeneity of variance. An ANOVA was performed with square root transformed swimming speed as the response and type of swimming behavior (relaxed, transverse, and strike) as the predictor variable. A Tukey HSD post hoc test was used for pairwise comparisons.

2.3. Results

2.3.1. Swimming speed

The results of the stereoscopic camera footage show how quickly lionfish move along the reef during three different behaviors, relaxed swimming, traverse swimming, and striking at prey. Many more relaxed swims were captured than traverse swims and strikes, as they were by far the most common of the swimming behaviors (Table 1). There was some overlap between the speeds of traverse swimming and relaxed swimming, but no overlap between strikes and any other type of swimming (Figure 6). The maximum and minimum speed for relaxed swimming were 82.19 mm s^{-1} and 15.82 mm s^{-1} respectively, with an average of 44.75 mm s^{-1} . The maximum and minimum speed for traverse swimming were 209.29 mm s^{-1} and 56.56 mm s^{-1} respectively, with an average of 138.99 mm s^{-1} . The maximum and minimum speed for strikes was 739.72 mm s^{-1} and 577.38 mm s^{-1} respectively, with an average of 625.44 mm s^{-1} .

Table 1. Types of speeds measured with their means, standard deviation, and number of occasions measured

	Mean (mm s^{-1})	Standard Deviation	n (Occasions measured)
Relaxed	44.75	15.46	25
Traverse	138.99	48.66	12
Strike	625.44	66.94	5

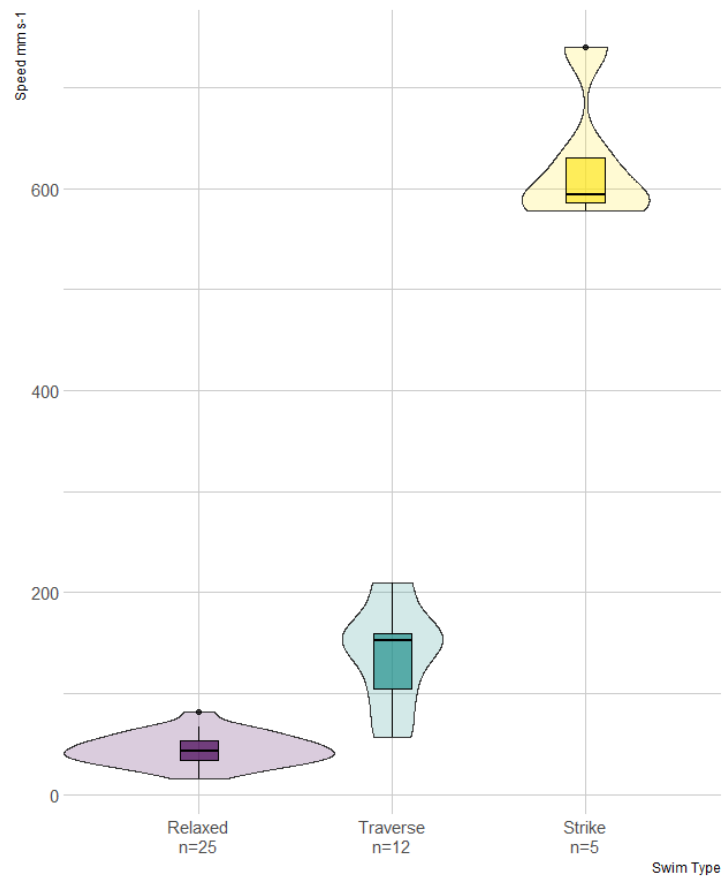


Figure 6. Violin boxplot of swimming speed. The width of the “violins” denotes the relative abundance of each observation were within that range. Small overlap occurs between relaxed and traverse, but neither overlap with strikes.

The ANOVA provided strong evidence for a significant difference among swimming speeds associated with each type of swimming behavior identified (Table 2). The post hoc analysis identified a difference in swimming speeds for all pairwise comparisons (relaxed versus traverse, $p < 0.0001$; relaxed versus strike, $p < 0.0001$; traverse versus strike, $p < 0.0001$).

Table 2. ANOVA table for swimming speed, Type = type of swimming behavior (relaxed, traverse, strike).

	DF	SUM SQ	MEAN SQ	F VALUE	p-VALUE
TYPE	2	1441.7	720.9	301.5	< 0.0001
RESIDUALS	39	93.2	2.4		

2.3.2. Lionfish behavior

The video footage captured during this study did include intraspecific aggression, but only a single case (Figure 7). Two adult lionfish of unknown sex pressed together and interlocked dorsal spines, swimming with high energy against each other for several minutes until one lionfish retreated, with both undergoing color changes during this period. Shortly after the initial retreat, the lionfish went out of frame only briefly, returning for a second round of the same behaviors. The lionfish did not reconvene after this second round. A third lionfish was present several meters behind the two aggressive lionfish. The third fish did not show any appearance of aggression and did not appear to acknowledge the behavior of the two aggressive lionfish in any way.



Figure 7. Lionfish aggression; note interlocking dorsal spines, pale coloration, and bodies angled toward each other.

Other behaviors included following conspecifics without apparent aggression, making similar turns and body positions with a short delay, aggregation for no obvious purpose, and hunting in close proximity for the same targets (Figure 5). The last behavior included several instances of lionfish going through typical hunting behaviors with flared fins but done close enough to be touching other lionfish at the same time, and seemingly tracking the same prey. This behavior was clearly obvious in a case which did not include hunting the population on the local patch reef where the lionfish were stationed. A school of several dozen fish of the family Carangidae swam quickly into frame clearly chasing a school of small prey fish with several

hundred individuals, which appeared to be juvenile *Haemulon flavolineatum* (French grunt). As soon as the lionfish noticed the school of prey fish, their body posture changed immediately from relaxed swimming to a hunting posture common right before striking, with all three portions of the pectoral fins flared vertically and the body posture angled towards the school, following it. Four lionfish could be seen in close proximity to each other (but with one occasionally falling behind) all with the same fin display and body posture following the school. Once the prey school was chased near rocks, a strike occurred from one lionfish, which did not appear to stop the hunting behavior of the other lionfish. The behaviors continued until they were no longer in the frame of the cameras.(Figure 5)

Within this study, a fear response was not noted when typical reef predators passed such as *Gymnothorax funebris* (green moray), several species of *Epinephelus* (groupers) large schools of the family *Carangidae* (jacks), or *Ginglymostoma cirratum* (Atlantic nurse shark). In this study, a fear response would be defined by fleeing in the opposite direction of the predator at a fast pace and hiding within structure or holes. A fear response was noted one time an unidentified sea turtle was in the camera frame. Several other instances of much closer approaches by sea turtles elicited no response, but included close inspection by the turtles of the cameras and the patch reef inhabited by lionfish. The most obvious fear response occurred at the end of filming when live capture of lionfish was initiated for further laboratory studies. No major fear responses were apparent before this time when diver interactions occurred.

2.4. Discussion

The lionfish in this study demonstrated three distinct swimming behaviors on coral reefs in the Florida Keys. Relaxed swimming, traverse swimming, and strikes all had significantly different speeds when compared to each other. There was minor overlap between outliers of relaxed and traverse swimming, but no overlap between strikes and any other behavior.

Previous literature on lionfish movement speed is primarily focused on large scale movements between areas of reefs on a time scale longer than this study, often by acoustic telemetry or tagging (Bachelier et al. 2015, Tamburello and Côté 2015, McCallister et al. 2018). One recent paper by Green et al. (2021) and a previous paper Green et al. (2011) provide literature values for small scale lionfish movement speed. The results of relaxed swimming speed (Average = 44.75 mm s^{-1}) in this study are close to the ranges presented in these previous studies, being somewhat higher than the Green et al. (2011) average of 2.46 mm s^{-1} (<https://automeris.io/WebPlotDigitizer>) and quite close to the average of tagged fish in Green et al. (2021) of 51 mm s^{-1} (high survival) and 63 mm s^{-1} (low survival). Green et al. (2021) also noted the several individuals who traveled distances $>1\text{km}$ had an average of 260 mm s^{-1} , which is closer to the maximum of the traverse swimming speed presented in this study at 209.28 mm s^{-1} . Both of these studies noted movement over longer periods (10 to 30 minutes) than this study, which measured individual behavioral movements over periods of <1 minute. Given the length of time in previous studies, it is likely that these were not straight line measurements as in this study, and they may have included multiple behaviors, such as relaxed swimming and hunting, within the measurement period. This study represents the first time that small scale lionfish movement speed during behaviors has been measured with the use of photogrammetry.

Lionfish typically exist in the environment either solitarily or in small groups, often cohabiting the same rock outcropping despite no apparent lack of available space (Fishelson et al. 1975, Kendall 1990). Social behaviors in lionfish such as social hunting, mating, and aggression have been observed, but are typically poorly studied (Fishelson 1975, Moyer and Zaiser 1981, Kendall 1990, Fogg and Faletti 2018). One example of a social behavior is aggression, which has been documented in invasive lionfish by Fogg and Faletti (2018) as well as appearing in short notes in other papers (Cure et al. 2012, Benkwitt 2016) and in their native range (Fishelson 1975, Moyer and Zaiser 1981). The case of aggression witnessed in the course of this study followed closely the description of aggressive behavior in lionfish from other sources: in short, two lionfish approached each other, pushed their bodies against each other with high intensity fin movements, horizontal head beatings, angling of dorsal spines, and mouth biting followed by color changes and the retreat of one of the fish (Fishelson 1975, Fogg and Faletti 2018, Moyer and Zaiser 1981). It is assumed that aggressive behavior is related primarily to fighting over a mate (Fishelson 1975, Moyer and Zaiser 1981).

Given the high abundance of lionfish on invaded reefs (Dahl and Patterson 2014, McTee and Grubitch 2014, Trégarot et al. 2015) one might expect that so many lionfish in such a small area would lead to an increase in aggressive behaviors as it does in other species such as brown trout (Kaspersson et al. 2010) and invasive crayfish (Pintor et al. 2009), but very few records or reports of aggression in invasive lionfish seem to exist despite the amount of hours between all the previous scientific studies spent observing them. However, other studies suggest that there may be a bell curve for aggression in certain species. Jones (1983) showed that juvenile wrasse, *Pseudolabrus celidotus*, had the highest rates of aggression at intermediate densities, and lower aggression at high and low densities. This could explain the low number of observations of

aggressive behavior in lionfish if most research is occurring in areas where lionfish densities are relatively high, but there are no studies of lionfish behavior in groups of varying densities to conclude that this may be the case.

Social hunting behavior in fishes has been studied in other species, with both interspecific and intraspecific cooperation taking place (Bshary et al. 2006, Vail et al. 2013, Vail et al. 2014, Steinegger et al. 2018). The idea of social hunting behaviors has been considered in species of lionfish, but results from studies have been varied with differing conclusions. Two studies (Lönstedt et al. 2014, Rizzari and Lönstedt 2014) noted cooperative hunting techniques used by *Dendrochirus zebra*, a species of small lionfish. These studies were called into question by an ethics board but were later cleared (The Biology Letters Editorial Team 2019). One study showed cooperative behaviors between *Pterois volitans* and *Gymnothorax griseus* (geometric moray) while hunting in seagrass meadows (Naumann and Wild 2013) but whether this is intentional active cooperation is unclear. Kendall (1990) observed what appeared to be cooperative foraging to herd schools of prey fishes in *Pterois miles* within their native range. A more recent study refuted the idea that lionfish pair up with conspecifics to assist with foraging (Sarhan and Bshary 2021), but this study involved observing lionfish with divers and snorkelers (which may have influenced behavior), and laboratory experiments which did not attempt to replicate natural conditions. Overall, there is some uncertainty still on if and how certain behaviors occur in lionfish populations, and even more uncertainty on how they may change with high population densities in the invaded range. It seems prudent to note these behaviors when they occur, even if they are rare or unclear in their origin, as doing so provides evidence of behaviors for future studies to draw upon.

This study did record behavior that would suggest a type of social hunting behavior similar to what Kendall (1990) described. Kendall (1990) visually observed multiple *Pterois miles* converge around a bait ball of prey fishes with outspread fins where they proceeded to strike one at a time, with each lionfish continuing to herd the prey with their pectoral fins even after they had fed. In our recording, lionfish reacted to a large influx of prey fish driven by larger predators in the Carangidae family. They followed a similar description to Kendall (1990), and multiple lionfish took up a hunting position with outspread pectoral fins, using them to follow and “herd” the school of prey. A strike occurred, and appeared to be successful, but none of the lionfish stopped following the school of prey and continued until out of the frame of the camera. While a communicative display of fins or body positioning similar to Lönnstedt et al. (2014) or Vail et al. (2013) was not observed, these lionfish had been near each other on previous days of camera deployments, and it is possible that they were already familiar with each other to some extent, thus reducing the need for communication.

A fear response in lionfish was not observed commonly during this study. On the rare occasion it was observed, it was strongest in reaction to divers, particularly when they got close to capture lionfish for later study in the laboratory. The response was closest in nature to the traverse swim noted in this study, with pectoral fins folded against the side of the body, dorsal spines folded down, and rapid beating of the caudal fin. This appears to be an attempt to streamline themselves to cover large distances quickly with minimal effort by reducing drag. Common reef predators, including eels, groupers, nurse sharks and jacks did not elicit this fear response in the lionfish. One *Gymnothorax funebris* (green moray) (estimated by the divers at 1.5 meters in length) and one *Gymnothorax moringa* (spotted moray)(estimated at 1 meter in length)

were present on the primary patch reef every day of observation, and there was no apparent response by the lionfish.

When considering future behavioral studies on lionfish in their invasive range, it may be useful to consider studies on similar species. Examples include Fishelson 1975 and Moyer and Zaiser 1981. These two studies provide an interesting, more complex dive into the behaviors of various *Pterois* and *Dendrochirus* (a very closely related genus) species, with attention given to how these behaviors influence individual movement around the immediate reef area. The behaviors studied included movements and aggregations for mating, feeding, and apparent small scale territories being established. Importantly, these studies also delve into some of the social organizations, such as hierarchies for mating, shown in the wild within their native ranges, which provides some context as to why certain behaviors like aggression are shown.

The data obtained in this study are useful in several areas, but primarily as a stepping stone for more accurate modeling to support management of invasive lionfish. Different behaviors are associated with different swimming speeds, and the swimming speed can be used in bioenergetics models to determine how energy is expended over time as well as how an individual fish approaches gaining energy (Price 1989, Boisclair and Sirois 1993, Brownscombe et al. 2014, Marras et al. 2015). Alone, swimming speed data is not enough to form a full model or plan, but when paired with other data and input into modeling efforts such as bioenergetics models (Cerino et al. 2013) and large scale acoustic telemetry and tagging (Bacheler et al. 2015, Tamburello and Côté 2015, McCallister et al. 2018) swimming speed data could be useful for planning how lionfish interact with their environment, and how might be best to target them for removal.

Invasive lionfish, despite the harm they cause to the environment, offer an excellent test case to assess hypotheses related to foraging theory, density-dependent behavior, the impacts of fear, and communication in fishes. In order to assess these hypotheses, future studies must provide fine scale monitoring for quantifying these behaviors. Measuring small behaviors such as fin flares, speed and distance of aggressive fish, body angles with respect to the substrate or other lionfish, and body and fin displays during mating are all possible with stereo-video systems that minimally affect natural behaviors. This will allow us to be able to have a greater understanding of how these behaviors can incorporate into the larger goal of modeling through bioenergetics models and eventually eradication efforts.

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Vita

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