An appetite for invasion: invasive lionfish have lower costs of digestion at high temperatures and a feeding physiology that may drive their impact

by

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Abstract

Species invasions threaten global biodiversity, but the physiological underpinnings of invasive characteristics are poorly understood. Specific Dynamic Action (SDA), the increase in metabolic rate associated with feeding and digestion, is a physiological process that strongly influences an animal's feeding ecology in concert with aerobic scope, or the range between its minimum and maximum physiologically possible metabolic rates. Both of these characteristics are temperature-dependent and may represent ecologically relevant responses to temperature changes. I investigated the relationship between SDA, aerobic scope, and temperature in lionfish (*Pterois spp.*), an invasive species of major concern to western Atlantic marine ecosystems. I collected lionfish from reefs in The Bahamas and used intermittent-flow respirometry to calculate their SDA and aerobic scope at two ecologically relevant temperatures (26° and 32° C). My results suggest that lionfish possess physiological traits that facilitate their invasiveness, and that they may benefit from warming ocean temperatures from climate change.

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Chapter 1. introduction

Species invasions and ecophysiology

Species invasions are a widely-recognized threat to global biodiversity, among which fish are highly represented with at least 624 known introductions (Gozlan 2008, Ricciardi et al. 2017). Invasive species have deleterious effects on their introduced ecosystems by removing native plant and animal species through predation or grazing, outcompeting native species, introducing diseases, and physically altering environments to the detriment of native species (Mooney and Hobbs 2000). While alien species are introduced to new ecosystems frequently, relatively few become established well enough to cause significant harm, and many are unable to persist (Williamson and Fitter 1996). The ecosystem- and community-level factors that contribute to an invasive species' success are well established, such as its niche, growth rate, reproductive capacity, and tolerance to disturbance (Van Kleunen et al. 2010), yet the physiological traits that underlie these factors have received relatively less attention. Understanding these traits and how they vary with the environment and among individuals can better inform the management of invasive species and predict how they will respond to a globally changing environment (Kelley 2014, Lennox et al. 2015).

Metabolic rate has been proposed as a potential determinant of invasion success, as it represents the net energy expenditure of numerous biological processes that could influence invasion success (McMahon 2002). Relatively little research has examined its relevance to invasive fish, however, despite fish comprising some of the most intensively-researched invasive species (but see Marras et al. 2015, Behrens et al. 2017, McCallum et al. 2017, Srean et al. 2017, Nati et al. 2018, Tessier et al. 2018). Resting metabolic rate has been compared between invasive species and their native counterparts in other taxa to determine the life history traits that drive invasion success (González-Ortegón et al. 2010, Lejeusne et al. 2014, Lagos et al. 2017). Metabolic rate may also be an important conduit through which climate change can affect fish species invasions, as it's strongly determined by temperature in ectotherms (Fry 1971, Maazouzi et al. 2011, Lejeusne et al. 2014). Many invasive species are adapted to thrive in disturbed or extreme conditions that will become more common with climate change, and the rate of successful species invasions is expected to increase in the future as a result (Mainka and Howard 2010). Despite this recognition within invasion science, temperature dependency of defining physiological traits like metabolic rate has been studied in few invasive species (Kelley 2014, Marras et al. 2015, Lennox et al. 2015). One such metabolic rate characteristic is Specific Dynamic Action (SDA): the postprandial increase in metabolic rate associated with feeding and digestion.

Specific Dynamic Action and its role in fish ecophysiology

SDA represents the postprandial increase in metabolic rate associated with feeding and digestion and is typified by a rapid rise in metabolic rate up to a short-lived peak followed by a longer decline (Secor 2009) (Fig. 1). McCue (2006) separates the mechanisms underlying SDA into three stages. Pre-absorptive pathways constitute the energy expended to catch, handle, and internally store or transport food to digestive organs, as well as the cost of enzyme and metabolite production. Absorptive pathways expend energy to absorb broken-down food through the intestinal lining and transport this absorbed product to other organs through the bloodstream. Post-absorptive pathways constitute the energy expended to transform and assimilate absorbed food product, including protein synthesis and amino acid production in cells throughout the body, ketogenesis in the liver, urea production, and excretion. Post-absorptive protein handling has been found to constitute the greatest proportion of energy expenditure in SDA, but the relative significance of each process influences the final nature of an SDA response and reflects differences in feeding ecology between species (Pannevis and Houlihan 1992, Eliason et al. 2008).

The cost and duration of SDA increases with the size of an ingested meal. The equivalent percentage of energy gained from a meal used to digest it—termed the SDA coefficient—may strongly influence foraging ecology and behavior, as ration-dependent assimilatory efficiency in gut tissues may select for meals sizes that minimize SDA coefficient and maximize energy gain (Jobling and Davies 1980, Carter and Brafield 1992, Fu et al. 2006). Most SDA studies in fish have analyzed single feeding events, however many fish may feed more frequently than the time required to fully digest a single meal. Ectotherms can down-regulate gut function to conserve energy during periods of low food availability or suppressed metabolism, but must then expend additional energy to up-regulate gut function upon feeding (Iglesias et al. 2003, Secor 2009). Frequent feeding may lower the cost of SDA by maintaining a high gut metabolism, but the biological significance of this would depend on the extent and frequency of gut down-regulation in a given species, which has been studied in few fish species (Ross et al. 1992, Fu et al. 2005, Zaldúa and Naya 2014).

As with all aspects of metabolism in fish, temperature is a strong determinant of SDA (Fry 1971). SDA in most ecotherms is characterized as being shorter in duration and higher in peak with increasing temperature, with the total cost of SDA unaffected (McCue 2006). Fish SDA has a more variable relationship to temperature, however, with a constant magnitude and cost of SDA but higher peaks and shorter durations with increasing temperature in some species (Jobling and Davies 1980, Machida 1981, Peres and Olivia-Teles 2001, Di Santo and Lobel

2015), an increasing magnitude, cost, or peak of SDA in others (Guinea and Fernandez 1997, Luo and Xie 2008, Peng et al. 2014, Khan et al. 2015, Di Santo and Lobel 2015, Tuong et al. 2018), a decrease in others (Cui and Wootton 1988, Garner et al. 1998), a non-linear variation in others (Pérez -Casanova et al. 2010, Tirsgaard et al. 2015), or a relationship that changes through ontogeny in others (Peck et al. 2005, Pérez -Casanova et al. 2010). The mechanistic underpinnings of these relationships are not fully understood, but temperature dependency in post-absorptive protein handling is believed to be responsible, as well as temperature dependency in Apparent Digestibility Coefficient (ADC), or the proportion of food energy assimilated versus excreted (Pannevis and Houlihan 1992, Hardewig and van Dijk 2003, Kofuji et al. 2005).

The relevance of these temperature effects, however, may ultimately depend on the relationship between SDA and its general energy metabolism. As SDA is a measure of metabolic rate, it's defined in relation to the lowest and highest possible metabolic rates in a fish—standard metabolic rate (SMR) and maximum metabolic rate (MMR), respectively. SMR is the minimum energy expenditure required for homeostasis and maintenance at rest and MMR is the highest biologically possible metabolic rate, defined by limits of cardiac performance and usually elicited following exhaustive exercise. The range between these limits is termed aerobic scope, and all aerobic energetic processes must occur in this range (Nelson and Chabot 2011, Jensen et al. 2017). SDA may occupy a large proportion of aerobic scope and is therefore an influential characteristic of a fish species' ecology. While eating large meals may be ecologically advantageous in that they maximize energy intake versus the energy cost and predation risk of foraging, they will simultaneously limit aerobic scope available for exercise, which is necessary for many ecologically-defining behaviors like predator avoidance, competition, foraging, and migration (Norin and Clark 2017). As such, a tradeoff occurs between meal size and maintaining aerobic scope, which may be important enough in some species to supersede predictions from optimal foraging theory (Lankford and Targett 1997, Jackson et al. 2004, Norin and Clark 2016). In turn, the relationship between SDA, SMR, MMR, growth, and behavior is believed to be an important determinant of a fish species' ecology and reflect selective pressures in its evolutionary history (Cutts et al. 2002, Fu et al. 2008, Norin and Clark 2016). These relationships may manifest through a fish's metabolic phenotype, or intraspecific variation in metabolic rate that can be as high as three-fold. Variation in metabolic phenotype has been found to correlate with a wide range of behaviors and life history traits that increase performance and fitness (Metcalfe et al. 2016), however only one study to date has examined its relationship to SDA. Millidine et al. (2009) found that juvenile Atlantic Salmon (*Salmo salar*) with high-SMR phenotypes had higher SDA costs but shorter SDA duration. In salmonids this is likely a tradeoff for faster growth, but the relationship between SMR, aerobic scope, and growth is species- and context-specific (Álvarez and Nicieza 2005, Auer et al. 2015). The relevance of temperature dependency in SDA will depend on its position in the energy budget defined by SMR and MMR, both of which can vary with temperature independently and either compound or mitigate the effect of temperature on SDA (Secor 2009, Auer et al. 2015, Metcalfe et al. 2016).

These dynamic metabolic relationships may be influential in species invasions. While predator-prey interactions may select for a tradeoff between meal size and aerobic scope, an invasive species lacking predators may be released from such constraints, and a lack of such a tradeoff would likely facilitate invasiveness (Jackson et al. 2004, Norin and Clark 2017). Determining an invasive species' SDA and aerobic scope across temperatures could determine whether their temperature optima covary and how this could affect its feeding ecology, giving insight to how species invasions may vary with climate change (Tirsgaard et al. 2015, Marras et al. 2015).

Invasive lionfish and their biology, ecology, and physiology

I used this approach to study SDA and its relationship to aerobic scope in invasive lionfish (*Pterois spp.*) (Fig. 1). Lionfish are mid-sized demersal predators native to the Indian and Pacific oceans that have been invasive to the western Atlantic Ocean since 2001, as well the Mediterranean since 2012 (Hixon et al. 2016, Bariche et al. 2017). Their phylogeny has recently been contested, so lionfish will be referred to generically henceforth (Wilcox et al. 2017). Based on their extant genetic diversity, Selwyn et al. (2017) determined that the western Atlantic founding population was as small as a few dozen individuals, supporting the hypothesis that lionfish were introduced by pet-owners releasing them from private aquaria. Western Atlantic prey species appear to be naïve to lionfish as a predation threat, and large predators generally don’t seem to recognize them as potential prey, allowing them to spread and forage with few, if any, limits (Albins and Hixon 2013, Valdivia et al. 2014, Anton et al. 2016). This may be due to a recently-discovered but as-yet unexplained ability to circumvent recognition in even their Indo-Pacific prey—possibly by masking their scent in combination with their cryptic morphology—which gives them exceptionally high strike success rates in both their natural and invaded range (Green et al. 2011, Cure et al. 2012, Lönnstedt and McCormick 2013, McCormick and Allan 2016). A high reproductive rate—spawning as frequently as every 4 days—and a pelagic larval stage fuels lionfish dispersal and maintains recruitment on invaded reefs (Morris et al. 2011, Côté and Green 2012). Invasive lionfish grow faster and larger than those in their native range, and can occur in densities almost five times greater (Green and Côté 2009, Whitfield et al. 2007, Pusack et al. 2016). As a result, lionfish have spread from Florida along the American eastern seaboard north to Rhode Island, throughout the entire Caribbean and Atlantic coast of Central America, and most recently to southern Brazil, making it one of the largest marine invasions to date (Kimball et al. 2004, Morris and Whitfield 2009, Sutherland et al. 2010, Ferreira et al. 2015).

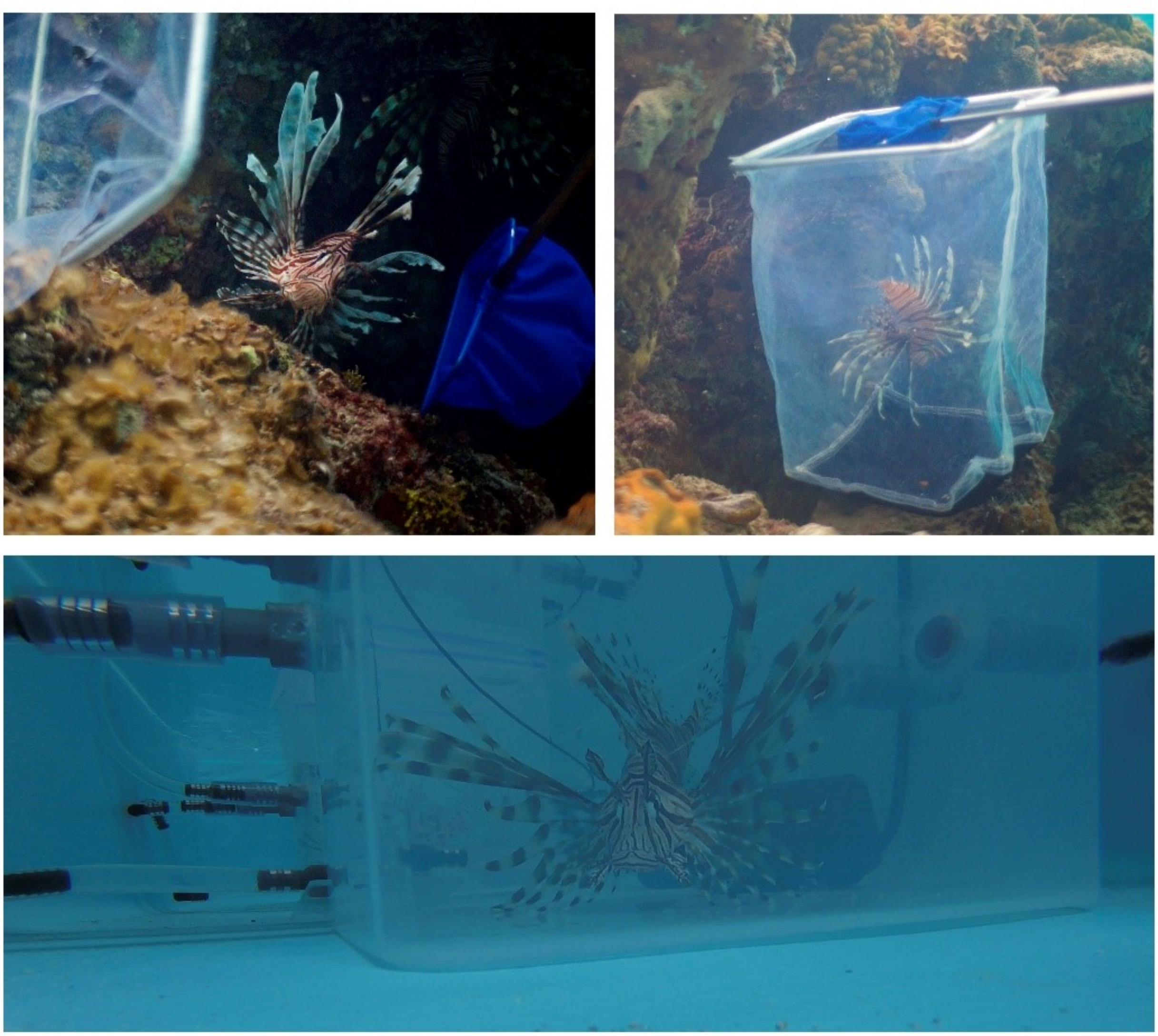

Figure 1. Invasive lionfish being captured from shallow patch reefs off Cape Eleuthera (top images) and in the respirometry chambers used in this study (bottom image). Top images used with permission from Ben Kaufman.

Invasive lionfish have deleterious effects on western Atlantic marine organisms. They prey on over 50 native species of small reef fish and crustaceans, as well as the juveniles of economically-important fishery species (Green et al. 2012, Dahl et al. 2017), and have been found to cause declines in abundance and density—even local extirpation—in numerous prey taxa (Albins and Hixon 2008, Green et al. 2012, Benkwitt 2015, Hixon et al. 2016, Palmer et al. 2016, Ingeman 2016, South et al. 2017). At least one species, Social Wrasse (*Halichoeres socialis*), is threatened with extinction by the lionfish invasion due its small range, however lionfish have been observed hunting undescribed and data-deficient species, so their full impact is likely not well understood (Rocha et al. 2015, Palmer et al. 2016, Tornabene and Baldwin 2017). Lionfish predation on small parrotfish can significantly decrease grazing pressure that prevents algae from outcompeting coral, which could stymie conservation efforts to reverse the phase shift from coral-dominated to algae-dominated reef community structure that occurred throughout the Caribbean in the late 20th century (Kindinger and Albins 2017, Albins and Hixon 2013). Such a phase shift has been observed more recently in mesophotic reefs, where the lionfish invasion was associated with declines in coral cover and reef fish diversity (Lesser and Slattery 2011, Dahl et al. 2017). Lionfish also have indirect effects on invaded reefs and other marine habitats. They occupy similar niches of native mesopredators and can effectively outcompete them, and can also increase native mesopredators' predation risk by physically expulsing them from reef crevices (Curtis et al. 2017, Raymond et al. 2015). Lionfish target small species that form cleaning mutualisms with other native species, resulting in higher parasite loads of resident fish on invaded reefs and lower visitation rates of transient fish and sea turtles (Tuttle et al. 2017). Invasive lionfish have also been found in mangrove, seagrass, and estuary nursery habitats, where their predation may impact community structure across marine ecosystems by limiting or preventing recruitment (Albins and Hixon 2013, Pimiento et al. 2015). Due to this array of deleterious effects, Sutherland et al. (2010) identified the lionfish invasion as one the largest individual threats to global biodiversity.

Much of the lionfish literature has focused on their feeding ecology, but data on the rate at which they consume native species is sparse. Green et al. (2011) estimated an adult individual could consume 8.9% of its body mass (BM) per day based on observations of wild lionfish in The Bahamas, which was more than twice the required daily energy intake estimated by Côté and Maljković (2010). Prey size selection and consumption rate of lionfish in aquaria have been found to differ with temperature, however, suggesting that this 8.9% BM per day rate may vary across seasons and thermal environments (Green et al. 2011, Cerino et al. 2013, South et al. 2017). Temperature-dependency in SDA could underpin such changes and affect lionfish foraging ecology in concert with temperature-dependency in aerobic scope (Auer et al. 2015).

A thermal scope of almost 25° C has allowed lionfish to invade a wide latitudinal range, as well as cold mesophotic reefs as deep as 100 meters (Dabruzzi et al. 2017, Tornabene and Baldwin 2017). Barker et al. (2017) found a temperature preferendum of 28.7° C in invasive lionfish from Florida, similar to the 29.8° C optimum for food consumption found by Cerino et al. (2013), however Dabruzzi et al. (2017) found a lower temperature preferendum between 23° and 24° C in Indo-Pacific lionfish. The mean CT_{min} and CT_{max} of lionfish—or the minimum and maximum temperature thresholds at which fish lose equilibrium, respectively—however, were similar between Dabruzzi et al. (CT_{min}=12.1° and CT_{max}=35.3° C) and Barker et al. (CT_{min}=12.1° and CT_{max}=36.5° C). Invasive lionfish therefore have a preferendum and consumption optimum close to their upper thermal threshold, a typical trait in tropical fish, however physiological metrics such as aerobic scope have not been tested in lionfish across temperatures to date

(Nillson et al. 2009, Norin et al. 2013, Rummer et al. 2014). Warming sea temperatures are anticipated to expand the lionfish's invasive northern and southern limits—currently set by lethally low winter temperatures—and to increase the habitability of temperature-structured ecosystems throughout their invaded range (Kimball et al. 2004, Whitfield et al. 2014, Bernal et al. 2015). Higher metabolic rates associated with increasing sea temperatures are predicted to shorten larval duration and increase feeding rates in invasive lionfish (Côté and Green 2012, Cerino et al. 2013), however SDA, aerobic scope, and their relationship to temperature have not been studied in lionfish to date.

Thesis objective

The objective of this study was to determine the relationship between invasive lionfish SDA and a) metabolic phenotype (SMR and aerobic scope), b) temperature (26° vs. 32° C), and c) feeding frequency (single vs. repeated feeding). These temperatures were selected to represent contemporary winter and extreme summer conditions in the subtropical western Atlantic, respectively, of which the latter will become more common with climate change (NOAA 2018).

Hypotheses

1) SDA magnitude, peak, time to peak, and duration will increase with meal size while SDA coefficient decreases due to greater assimilatory efficiency in gut tissues.
2) Given that lionfish optimally feed close to their upper temperature thresholds, SDA cost and duration will decrease with temperature while SDA peak increases due to the higher metabolic demands of increased temperature.
3) Lionfish with higher SMR and larger aerobic scope will have a greater SDA cost but shorter duration as per the findings of Millidine et al. (2009).

4) Aerobic scope will decline with increasing temperature as it does in many coral reef fish species, with SDA occupying a greater proportion of it in turn.
5) The SDA cost of repeated feeding is lower than a single feeding due elevated gut metabolism and efficiency.
6) Peak SDA of a repeated meal will be higher when the previous meal is larger and more recent.
7) Lionfish will eat larger repeated meals when residual metabolic rate from the previous meal's SDA is lower, and when the previous meal is smaller and less recent.

Chapter 2. Methods and Results

2.1 Methods

Animal collection and husbandry

Lionfish ($\bar{x}$=135.5±9.5 g, all reported errors are standard error) were collected on SCUBA with plastic hand nets from patch reefs in the Bight of Rock Sound (24° C50’28 N, 76° C17’13 W) in the winter (January-March) and summer (June-July) of 2017 and winter of 2018 (Jan-Feb). Average ambient sea temperatures for the region were 24°, 29°, and 24° C during these collection periods, respectively (NOAA 2018). All collections were from less than 4 meters depth to prevent barotrauma in collected fish. In transport, lionfish were held in aerated coolers that had roughly half of their water exchanged every half hour. Fish were transported to the Cape Eleuthera Institute wet lab and held in circular 750-liter tanks that were aerated and continuously supplied with fresh seawater (5 L min^{-1}) at ambient temperature. All fish were acclimated to lab conditions for a minimum of 5 days and held for a maximum of 22 days before measuring metabolic rate, and were fed live silversides (*Atherinomorus stipes*) *ad libitum* every 3 to 5 days.

Respirometry

An 8-chambered intermittent-flow respirometry system (Loligo Systems, Hobro, Denmark) was used to measure oxygen uptake rates in individual lionfish in units of MO_2, the mg of O_2 consumed per kg of fish per hour (mg^{-1} kg^{-1} hr^{-1}). Chambers were custom-made from 10.15 L polypropylene containers (Snapware, Rosemont, USA) and plumbed with vinyl tubing to 5 L min^{-1} recirculation pumps and 10 L min^{-1} flush pumps bifurcated between 2 chambers for an effective flush rate of 5 L min^{-1} (Eheim, Postfach, Germany). The chamber lids had 3 cm ports sealed with rubber stoppers through which prey fish could be fed *in situ* during measurements. Chambers were immersed in 2 570 L raceways (300 x 30 x 60 cm) supplied with filtered and aerated seawater. Oxygen probes (Loligo Systems, Hobro, Denmark) were calibrated before each measurement period to 0% and 100% air saturation using a seawater-sodium sulphite solution and air-saturated seawater, respectively. MO_2 was recorded during a 10-min recirculation period, preceded by a 19-min flush period and 1-min wait interval. Microbial background respiration was recorded in each chamber for 3 measurement cycles (90 min) before and after each round of respirometry.

Experimental protocol

A single-feeding experiment was conducted at 26° (n=16) and 32° C (n=22), and a repeated-feeding experiment was conducted at 26° C (n=13). 26° C was chosen as it was the ambient winter sea temperature during this study and is representative of contemporary winter temperatures in the subtropical western Atlantic. 32° C was chosen as it is representative of high summer sea temperatures both in The Bahamas and in the invasive lionfish range nearest the equator (NOAA 2018), and because Cerino et al. (2013) observed a reduced consumption rate at 32° C.

In both experiments, fish were fed *ad libitum* in their holding tanks and then fasted for 48 h prior to respirometry to ensure a post-absorptive state. Fish were transferred directly from their holding tank into respirometry chambers and MO_2 was recorded for 24 h to calculate standard metabolic rate (SMR, see below). Fish were then fed live silversides *ad libitum* directly in their chambers during crepuscular hours, and MO_2 was immediately recorded to calculate specific dynamic action (SDA, see below). In the single-feeding experiment, SDA was recorded over 96 h following a single feeding that occurred between 17:00 and 19:00. In the repeated-feeding experiment, SDA was recorded for 72 h while fish were fed *ad libitum* every morning between

06:00 and 08:00 and every evening between 17:00 and 19:00, followed by 60 h without feeding. Rations ranged between 0.6% and 13.8% body mass (1.0-20.4 g). Maximum metabolic rate of activity (MMR_{chase}) was determined at the end of each trial by chasing each fish in a 150 L tank to exhaustion, determined when their flight reflex was impaired and the caudal fin could be held and let go three times in quick succession. The fish were then immediately returned to their respirometry chambers and MO_2 was recorded.

For the single-feeding experiment, the 26° C treatment was recorded in March 2017 and June 2017, and the 32° C treatment was recorded in July 2017. There was no significant difference in fish mass or SMR (see below) between the 26° C treatment measured in March and June according to Welch's t-tests, and there was no consistent temporal change in SMR or scope for activity (see below) between measurement rounds in either treatment as expected from an acclimation effect (Sandblom et al. 2014). All lionfish were used to measure SDA once, except for 3 lionfish used twice in the 26° C treatment in March and 5 lionfish used in the 26° C treatment in July that were used again in the following 32° C treatment.

All work was carried out under the Bahamas Department of Marine Resources permit number MAMR/FIS/17 and with approval from the Canadian Council of Animal Care and Carleton University. As they are a harmful invasive species, lionfish were euthanized after experimentation with cerebral percussion.

Feed fish calorimetry

The gross energy content of 10 silversides (*A. stipes*) were determined using an oxygen bomb calorimeter (Parr Instrument Co., Moline, USA). Their average gross energy density was 13.79±0.87 kJ g^{-1} and ranged between 8.85 and 17.21 kJ g^{-1}, similar to the energy density of demersal western Atlantic reef fish that lionfish would prey upon (Schwartzkopf and Cowan 2017, Welicky et al. 2018).

Data analysis

Raw MO_2 was corrected for background respiration for each chamber in each trial separately. Background respiration was calculated for each chamber based on a 1st-order exponential trendline calculated between initial and ending average background measurements, then subtracted from each MO_2 measurement slope to calculate background-corrected MO_2. Per Chabot et al. (2016), the minimum r^2 to ensure linearity of the oxygen trace slope was determined for each fish and values below this threshold were rejected, with an absolute minimum threshold of 0.80. The average r^2 across all fish was 0.96, and the fish with the lowest average r^2 was 0.89.

Background-corrected MO_2 was blocked by minimum values per every 4 measurements (2 hrs) to account for short bouts of activity in some fish per Eliason et al. (2007). Maximum metabolic rate at chase (MMR_{chase}), peak postprandial MO_2 (SDA_{peak}), and time-to-peak ($SDA_{peak\text{-}hr}$) were derived from unblocked MO_2. Standard metabolic rate (SMR), routine metabolic rate (RMR), and SDA_{total} were derived from MO_2 block minimums. SMR was calculated as the average of the lowest 10th percentile of MO_2 recorded over 24 h before feeding. RMR was calculated as the average MO_2 of the 18 h before feeding after a 6-h recovery period following placement in the respirometer. SDA duration (SDA_{dur}) was the number of hours between feeding and the third point of postprandial MO_2 to fall below RMR. Five feedings in the single-feeding experiment had postprandial MO_2 that didn't return below RMR within 96 h but were trending downward, for which their duration was extrapolated to the slope of the last period of declining MO_2 derived from a 5th-order polynomial trendline fitted to that feeding's SDA response. SDA_{total} was calculated by integrating the area under the curve of postprandial MO_2 over the duration of SDA minus SMR (Fig. 2A). SDA_{peak} exceeded MMR_{chase} in many fish and precluded a reliable measurement of true aerobic scope, so scope for activity was calculated as the difference between MMR_{chase} and SMR. The cost of SDA as a percentage of energy consumed is termed the SDA coefficient (SDA_{coeff}). This was calculated with the equation $SDA_{coeff}=(E_{SDA}/E_{meal}) \cdot 100$, where E_{SDA} is the energy spent on SDA assuming 1 g of O2 is associated with the release of 13.6 kJ of energy (Cho et al. 1982) and E_{meal} is the energy of an ingested meal, calculated by multiplying its mass by the average gross energy density we found in our feed fish (13.79 kJ g^{-1}) and a 0.8 correction factor to account for indigestible energy (Craig et al. 1978, Jobling 1983). Single values for SDA_{total}, meal size, and SDA_{coeff} across repeated meals were determined by integrating under the curve of all repeated meals, summing repeated meal sizes, and using these values to calculate SDA_{coeff} as above, respectively (Fig. 2B).

Body mass (BM) varied 7.6-fold in the single-feeding experiment (43.5-331.5 g, $\bar{x}$=140±11.3 g) and 3.5-fold in the repeated-feeding experiment (44-155 g, $\bar{x}$=92.3±9.9 g). Log10-transformed whole SMR (mgO2 hr-1) varied allometrically when regressed against log10-transformed BM (kg) at both temperatures used in this study. MO_2 was therefore mass-adjusted to that of a 140 g fish using the equation $y_{0.14\ kg}=y_M(M \cdot 0.14\text{-}1)^{(1\text{-}b)}$, where $y_{0.14\ kg}$ is Oxygen consumption rate mass-adjusted to a 140 g fish, y_M is Oxygen consumption rate (MO_2) of a fish at mass M, and b is the allometric scaling coefficient (Rosewarne et al. 2016). The scaling coefficient was almost identical between temperatures (b=0.83 at 26° C and b=0.84 at 32° C), so was averaged for all fish (b=0.835). Findings are presented for mass-adjusted data.

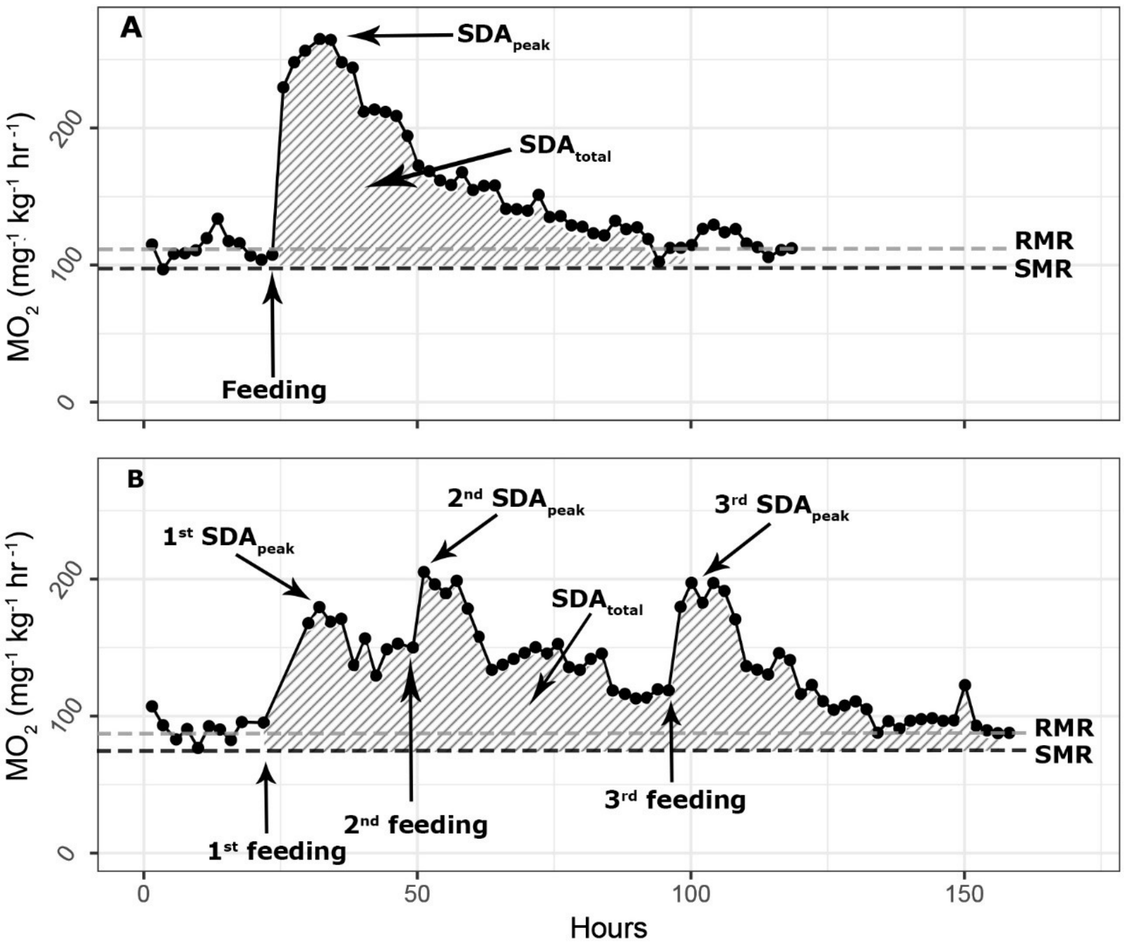

Figure 2. Examples of specific dynamic action (SDA) curves during single feeding (A) and repeated feeding (B). Total SDA (SDA_{total}) is the integral under the curve between postprandial MO_2 and standard metabolic rate (SMR, black line) over the duration between feeding and the third postprandial MO_2 value to fall below routine metabolic rate (RMR, grey line). Peak SDA (SDA_{peak}) is the highest unblocked postprandial MO_2 value following feeding. The single feeding curve was in response to a 7.4% body mass (BM) meal, and the first, second and third repeated feeding curves were in response to 3.2%, 2.7%, and 2.7% BM meals, respectively.

Statistical analysis

In the single-feeding experiment, due to high collinearity in the predictor variables, random-forest algorithms were used to assess the importance of meal size, temperature, SMR, and scope for activity as predictors of SDA_{total}, SDA_{peak}, $SDA_{peak\text{-}hr}$, SDA_{dur}, and SDA_{coeff} (Table

1). Random forests are a type of machine learning algorithm that fit a series of data trees via recursive binary partitioning with subsets of randomly chosen data, and are robust to traditional statistical assumptions (Breiman 2001). These determined the overall variance explained for each SDA parameter by the predictors, and the relative importance of each predictor to that relationship as indicated by the highest increases in mean-square error. The models were implemented with the randomForests package (Liaw and Wiener 2002) in R with tree number set to 1,000. All statistical tests were performed using R (R Core Team 2018, version 3.4.4) in Rstudio (Rstudio Inc, Boston, USA).

To further explore the relationships between the above predictor and response variables, separate ANCOVA models were fit for each SDA parameter to test the effect of temperature, meal size, SMR, and scope for activity (Table 1). All but two SDA parameters were normally distributed, homoscedastic, and met assumptions of ANCOVA, with SDA_{coeff} and $SDA_{peak\text{-}hr}$ log10-transformed and square-root transformed, respectively, to meet assumptions. Predictors were entered in the models in order of their relative importance from the random-forest algorithm, and only if there was a linear relationship between a predictor and the response variable. Interactions were determined by including interaction terms between temperature and each covariate in the ANCOVA model. If an interaction was present, main effects were analyzed between the temperature treatments separately, and Akaike's Information Criteria (AIC) was used to determine the optimal combination of predictors for each linear model. Relationships between the predictors were also analyzed to further explore the nature of their collinearity, using Welch's t-tests or regression.

Data from the repeated-meal experiment were analyzed in 3 separate ways, each accounting for the effect of meal size, SMR, and scope for activity as in the single-feeding experiment (Table 1). The first used multiple regression to determine whether meal size selection of a repeated meal was affected by residual SDA from the previous meal—measured as the percentage of scope for activity occupied by pre-feeding MO_2—as well as the previous meal's size, the previous meal's SDA integral, and the time interval since the previous meal. The second used multiple regression to analyze the effect of the previous meal's size, the previous meal's SDA integral, and the time interval since the previous meal on SDA_{peak} and $SDA_{peak\text{-}hr}$. Variance inflation factors were below 3. AIC was used to determine the optimal combination of predictors for each linear model. The third used ANCOVA models to test the effect of repeated- versus single-feeding on SDA_{total} and SDA_{coeff} while controlling for meal size, SMR, and scope for activity as covariates. SDA_{total} was log10-transformed to meet assumptions. To control for the effect of meal size, SMR, and scope for activity, these predictors were entered into each model first and in order of their importance from the random-forest algorithm in the single-feeding experiment, followed by the repeated-feeding predictors.

Table 1. Predictors and response variables in each statistical test used in this study.

Test	Predictors	Response variables
Single-feeding	Meal size Temperature SMR Scope for activity	SDA_{total} SDA_{peak} $SDA_{peak\text{-}hr}$ SDA_{dur} SDA_{coeff}
Repeated meal size	Residual SDA Previous meal size Previous meal SDA integral Time since previous meal Scope for activity SMR	Repeated meal size
Repeated-feeding	Previous meal size Previous meal SDA integral Time since previous meal Current meal size Scope for activity SMR	SDA_{peak} $SDA_{peak\text{-}hr}$
Single vs. repeated feeding	Feeding type Summed meal size Scope for activity SMR	SDA_{total} SDA_{coeff}

2.2 Results

Single-feeding experiment: relationships between temperature, meal size, SMR, and scope for activity

Lionfish fed *ad libitum* ate significantly more (P=0.001) in the 32° C treatment than the 26° C treatment, with a 65% increase in prey consumption from 26° C ($\bar{x}$=4.6±0.60% BM, 1.8-13.8% BM) to 32° C ($\bar{x}$=7.6±0.62% BM, 1.4-13.5% BM) (Fig. 3A). $\mathrm{MMR}_{\mathrm{chase}}$ did not significantly vary with SMR (Fig. 3B), and meal size selection did not significantly vary with SMR or scope for activity.

SMR significantly differed between temperatures (P<.001), with a 71% increase from the 26° C treatment ($\bar{x}$=66.5±3.7 mg^{-1} kg^{-1} hr^{-1}) to the 32° C treatment ($\bar{x}$=114.2±3.6 mg^{-1} kg^{-1} hr^{-1}), while $\mathrm{MMR}_{\mathrm{chase}}$ did not significantly differ between the 26° C treatment ($\bar{x}$=252.9±6.7 mg^{-1} kg^{-1} hr^{-1}) and the 32° C treatment ($\bar{x}$=257.1±7.9 mg^{-1} kg^{-1} hr^{-1}). Scope for activity significantly differed between temperatures (P=0.001), declining 23% from the 26° C treatment ($\bar{x}$=186.4±5 mg^{-1} kg^{-1} hr^{-1}) to the 32° C treatment ($\bar{x}$=142.9±8.2 mg^{-1} kg^{-1} hr^{-1}) than the (Fig. 5).

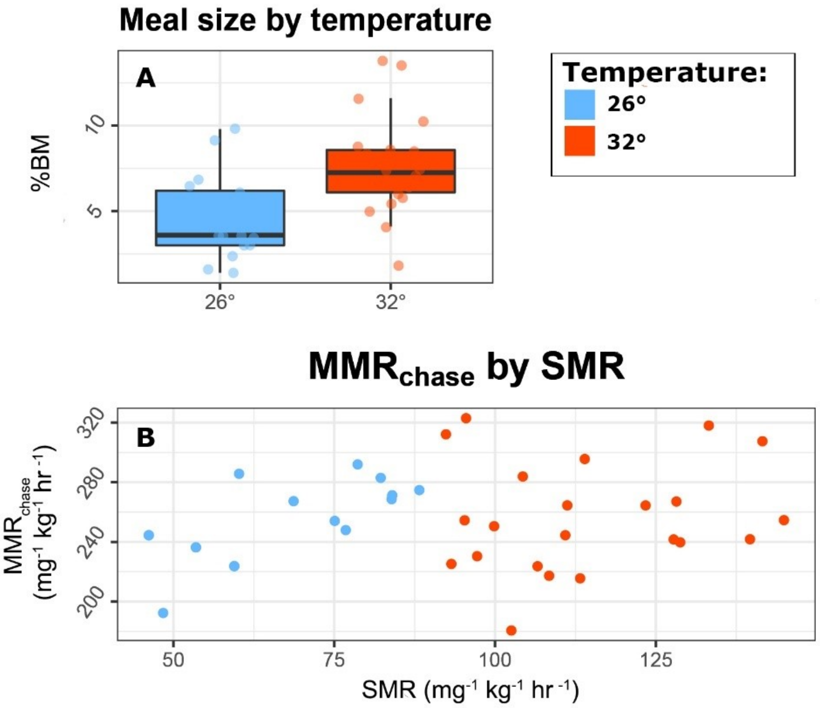

Figure 3. Relationships between meal size, temperature, maximum metabolic rate at chase (MMR$_{chase}$), and standard metabolic rate (SMR). Meal size is measured as a percentage of body mass (%BM). SMR and MMR$_{chase}$ are Oxygen consumption rate values (MO$_2$, mg^{-1} kg^{-1} hr^{-1}).

Single-feeding experiment: effects of temperature, meal size, SMR, and scope for activity on SDA parameters

Random-tree algorithms found that the predictors (temperature, meal size, SMR, and scope for activity) explained 66.2% and 11.1% of the variance in SDA$_{peak}$ and SDA$_{total}$, respectively, and that SDA$_{dur}$, SDA$_{peak\text{-}hr}$, and SDA$_{coeff}$ were poorly explained by the predictors (supplementary materials, Table 2).

SDA$_{peak}$ significantly increased with meal size (P<.001) and significantly differed between 26° C and 32° C (P<.001) (Figs 4A, 4D). When divided by meal size to control for its effect, SDA$_{peak}$ was 24% lower at 32° C than 26° C (Fig. 4D). SDA$_{peak}$ significantly increased with SMR (P=0.001) but not scope for activity (Fig. 4B). $\mathrm{SDA}_{\text{peak-hr}}$ did not significantly differ with temperature, meal size, scope for activity, or SMR ($\bar{x}$=6.4±0.77 hrs) (supplementary materials, Fig. 8).

Temperature had no significant effect on $\mathrm{SDA}_{\text{total}}$, $\mathrm{SDA}_{\text{coeff}}$, and $\mathrm{SDA}_{\text{dur}}$, however it interacted significantly or approaching significance with the effect of scope for activity (P=0.09, P=0.002, P=0.006, respectively), precluding the determination of its effect (Figs 4G, 4K, 4O). Main effects for these SDA parameters were therefore analyzed between temperature treatments separately.

$\mathrm{SDA}_{\text{total}}$ significantly increased with meal size in both temperature treatments (26° C: P=0.009; 32° C: P<.001) (Fig. 4E). $\mathrm{SDA}_{\text{total}}$ did not significantly differ with SMR or scope for activity in either treatment (Figs 4F, 4G). When divided by meal size to control for its effect, $\mathrm{SDA}_{\text{total}}$ was 25% lower at 32° C ($\bar{x}$=696.5±39.6 mg^{-1} kg^{-1} hr^{-1} $\%\mathrm{BM}^{-1}$) than 26° C ($\bar{x}$=932.4±122.9 mg^{-1} kg^{-1} hr^{-1} $\%\mathrm{BM}^{-1}$), but the interaction between temperature and scope for activity precluded determining the effect of temperature (Fig. 4H).

Average $\mathrm{SDA}_{\text{coeff}}$ was 14.1±1.3% on average and ranged from 2.2% to 36%. $\mathrm{SDA}_{\text{coeff}}$ significantly increased with scope for activity, but only in the 26° C treatment (P=0.02) and with relatively low predictive power (r^2=0.33). $\mathrm{SDA}_{\text{coeff}}$ did not differ with SMR in either treatment. $\mathrm{SDA}_{\text{coeff}}$ significantly decreased with meal size in the 32° C treatment only (P=0.024) and with low predictive power (r^2=0.14) (Figs 4I, 4K, Table 3). $\mathrm{SDA}_{\text{coeff}}$ was similar between 26° C ($\bar{x}$=14.5±2.4%) and 32° C ($\bar{x}$=14.1±1.3%), but the interaction between temperature and scope for activity precluded determining the effect of temperature (Fig. 4L).

SDA_{dur} ranged from 27.9 to 109.6 h, did not differ with meal size or SMR in either treatment, and significantly increased with scope for activity but only in the 26° C treatment (*P*=0.006) (Figs 4M-4O). SDA_{dur} was better explained by variability in SDA_{total} rather than meal size (*P*<.001 in both temperature treatments), with longer durations associated with larger SDA_{total} (supplementary materials, Fig. 9).

SDA occupied the entirety of scope for activity (i.e. $SDA_{peak}>MMR_{chase}$) in 72% of the feedings at 32° C, versus 63% at 26° C (Fig. 5). All meal sizes created SDA_{peak} that occupied more than 64% of a fish's scope for activity, however considerable variation in remaining scope for activity existed between similar-sized meals. Our equation for SDA_{peak} predicts meal sizes of 8.7% and 5% BM would occupy the entirety of scope for activity at 26° and 32° C, respectively, (i.e. $SDA_{peak}=MMR_{chase}$) (supplementary materials, Table 3).

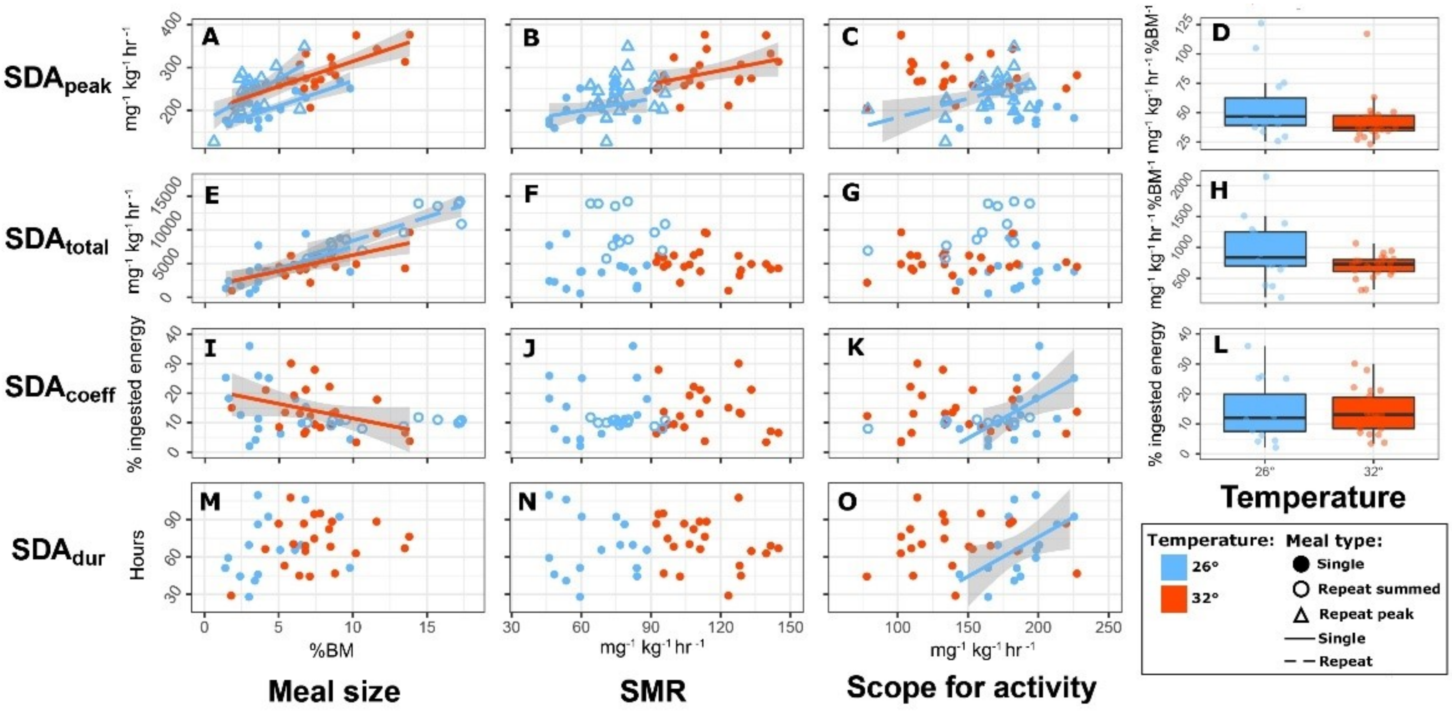

Figure 4. Relationships between specific dynamic action (SDA) parameters, temperature (26° and 32 ° C), standard metabolic rate (SMR), and scope for activity in both single-feeding (solid points and trendlines) and repeated-feeding (empty points and dashed trendlines) experiments. Trendlines with 95% CI included for significant relationships, however the significant relationships between meal size and SDA coefficient ($\mathrm{SDA}_{\mathrm{coeff}}$) and scope for activity and $\mathrm{SDA}_{\mathrm{coeff}}$ had relatively low predictive power (r^2=0.14 and r^2=0.33, respectively). Data for peak SDA ($\mathrm{SDA}_{\mathrm{peak}}$) and total SDA ($\mathrm{SDA}_{\mathrm{total}}$) in response to temperature (D and H) divided by meal size to control for its effect. Meal size is measured as a percentage of body mass (%BM). Scope for activity is the difference between maximum metabolic rate at chase ($\mathrm{MMR}_{\mathrm{chase}}$) and SMR in an individual lionfish. SDA coefficient ($\mathrm{SDA}_{\mathrm{coeff}}$) is the percentage of energy consumed in a meal spent on $\mathrm{SDA}_{\mathrm{total}}$, using the average gross kJ g^{-1} of our silversides multiplied by a 0.8 correction factor to account for indigestible energy. SDA duration ($\mathrm{SDA}_{\mathrm{dur}}$) is the length of time over which $\mathrm{SDA}_{\mathrm{total}}$ was derived, starting with feeding and ending with the third postprandial MO_2 value to fall below routine metabolic rate (RMR).

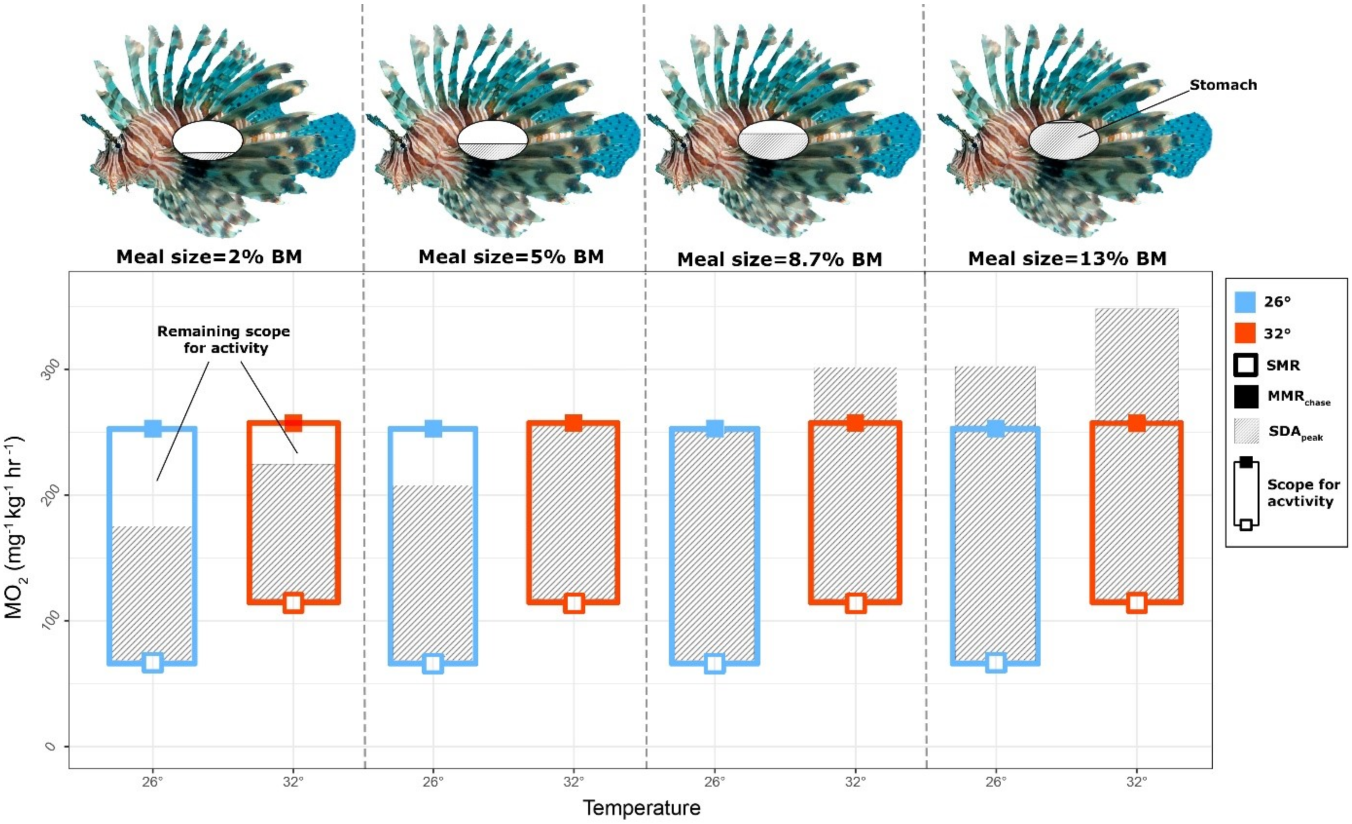

Figure 5. Relationships between peak specific dynamic action (SDA$_{peak}$), scope for activity, and temperature (26° and 32 ° C) as a function of meal size. Meal size is measured as a percentage of body mass (%BM). Standard metabolic rate (SMR) and maximum metabolic rate at chase (MMR$_{chase}$) are Oxygen consumption rate values (MO$_2$, mg^{-1} kg^{-1} hr^{-1}). Plotted SMR and MMR$_{chase}$ values are averages from each temperature treatment. Scope for activity is the difference between MMR$_{chase}$ and SMR in an individual fish. Lionfish that consume a small meal (2% BM) retain 42% of their scope for activity at 26° C on average, but only 23% at 32° C. SDA$_{peak}$ of a medium-sized meal (5% BM) fully occupies scope for activity at 32° C but leaves 23% of scope for activity at 26° C free. Scope for activity is fully occupied by SDA$_{peak}$ of an 8.7% BM meal at 26° C and is exceeded 1.3 times at 32° C. SDA$_{peak}$ of the largest meal size we observed (13% BM) exceeds scope for activity 1.3 times at 26° C and 1.6 times at 32° C.

Repeated-feeding experiment: effects of previous meals on meal size selection and $\mathrm{SDA_{peak}}$

Twenty-seven repeated meals were recorded across 13 lionfish, which ate between 2 and 5 meals each. Nine of these were eaten within 12 h of the previous meal, 8 were eaten within 24 h, and 10 were eaten within 36 to 60 h.

Repeated meal size selection ranged from 0.6% to 8.5% BM ($\bar{x}$=3.7±0.3%). The previous meal's size, SDA integral, time, or residual SDA had no relationship to meal size selection, nor a fish's SMR or scope for activity (Figs 6A-6D). $\mathrm{SDA_{peak}}$ significantly increased with meal size ($P<.001$), shorter intervals between meals ($P=0.02$), and larger scope for activity ($P<.001$), and was not significantly affected by SMR, previous meal size, or previous meal SDA integral (Figs 4A-4C, 6F-6H). $\mathrm{SDA_{peak\text{-}hr}}$ did not differ with meal size, the size or SDA integral of the previous meal, time since the previous meal, SMR, or scope for activity (supplementary materials, Fig. 8).

Repeated-feeding versus single-feeding

$\mathrm{SDA_{total}}$ across repeated meals did not significantly differ from that of single meals after the effect of meal size ($P<.001$) was accounted for (Figs 6E, 5E). As in the single-feeding experiment, $\mathrm{SDA_{total}}$ did not differ with SMR or scope for activity (Figs 4F, 4G). $\mathrm{SDA_{coeff}}$ across repeated meals was not significantly affected by meal size, SMR, or scope for activity (Figs 4I-4K). Heterogeneity of variance in $\mathrm{SDA_{coeff}}$ between single and repeated meals precluded analyzing it in an ANCOVA model, so it was assessed qualitatively. When divided by scope for activity to control for its effect, $\mathrm{SDA_{coeff}}$ of repeated meals ($\bar{x}$=11.6±1%) was slightly smaller than that of single meals on average ($\bar{x}$=14.5±2.4%), however $\mathrm{SDA_{coeff}}$ of single meals had much higher variability ($\sigma_{\bar{x}}$=5.3%) than that of repeated meals ($\sigma_{\bar{x}}$=1.9%) (Fig. 6I).

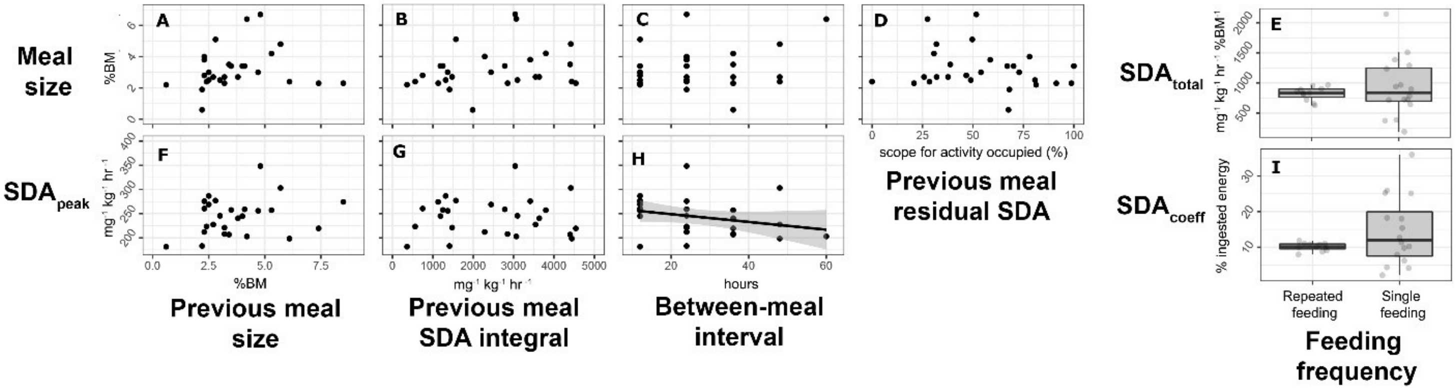

Figure 6. Relationships between specific dynamic action (SDA), meal size selection, and variables used in the repeated-feeding experiment (A-D and F-H), as well as the relationship between total SDA (SDA$_{\text{total}}$), SDA coefficient (SDA$_{\text{coeff}}$), and feeding frequency (E and I). Trendlines with 95% CI included for significant relationships. SDA$_{\text{total}}$, peak SDA (SDA$_{\text{peak}}$), and SDA$_{\text{coeff}}$ are Oxygen consumption rate values (MO$_2$, mg^{-1} kg^{-1} hr^{-1}). Data for SDA$_{\text{total}}$ in response to temperature (E) divided by meal size to control for its effect. Meal size is measured as a percentage of body mass (%BM). SDA$_{\text{coeff}}$ is the percentage of energy consumed in a meal spent on SDA$_{\text{total}}$, using the average gross kJ g^{-1} of our silversides multiplied by a 0.8 correction factor to account for indigestible energy. Previous meal residual SDA is the percentage of scope for activity occupied by SDA at the time of a repeated meal.

Chapter 3. Discussion

I found several lines of evidence for lionfish having physiological traits that could facilitate their invasiveness, and that these traits may make them robust to warming temperatures with climate change. I observed lionfish routinely attain postprandial metabolic rates that exceeded their scope for activity, which to date has been described in only one other fish species, Southern Catfish (*Silurus meridionalis*) (Fu et al. 2008). Furthermore, the amount of prey a lionfish consumed was independent of how much of its scope for activity was occupied by residual postprandial metabolic rate from its previous meal, or how large or recent that previous meal was. This coincides with the finding that even the smallest meals (<2.5% BM) occupied at least 66% of scope for activity at 26° C and 80% at 32° C and suggests that lionfish defensive morphology (i.e. venomous spines) allows them to feed without a clear metabolic tradeoff to the locomotory capacity other species need to avoid predators during SDA (Millidine et al. 2009, Norin and Clark 2017). Invasive lionfish forage generally on abundant and naïve native prey, and their success as an invasive species is attributed in large part to their ability to assimilate excess energy and allot it to rapid growth and frequent reproduction (Côté and Maljković 2010, Albins and Hixon 2013, Chappell and Smith 2016). The low-mobility, high-feeding physiology I found could mechanistically underpin this ability—as well as their high site fidelity, apathy toward potential predators, and tendency to forage until prey species are locally suppressed or extirpated—and explain how invasive lionfish sustain the high feeding rates required for their persistence and dispersal (Green et al. 2011, Tamburello and Côté 2015, Hixon et al. 2016, Ingeman 2016).

I may therefore hypothesize that lionfish foraging is primarily constrained by their absolute MMR, equivalent to SDA_{peak} of the largest possible consumable meal. My largest observed meal may have approached this at 13.8% BM, which produced an SDA_{peak} 1.7 times greater than that fish's scope for activity. This hypothesis, however, is at odds with the 8.9% BM day^{-1} feeding rate estimated by Green et al. (2011) in wild lionfish in The Bahamas. A single 8.9% BM meal would produce an SDA_{peak} only marginally above my average observed scope for activity at 26° C (Fig. 5), and in reality this daily rate would likely be spread across smaller meals with lower SDA_{peak} during morning and evening crepuscular hours (Côté and Maljković 2010, Green et al. 2011). In other words, based on the observations that lionfish a) feed independently of their scope for activity and the size or time of their previous meal and b) can greatly exceed their scope for activity during SDA_{peak}, a higher foraging rate would be expected in wild lionfish. This suggests that either Green et al.'s foraging rate was underestimated or that factors other than metabolic constraints mediate lionfish foraging. Selection for defensive morphology in animals is higher when food is scarce, necessitating longer foraging times with greater predation risk (Bennet et al. 2013, Steiner and Pfeiffer 2006). Food availability also strongly selects for foraging behavior, so my finding that Green et al.'s estimated 8.9% BM day^{-1} feeding rate is lower than physiologically predicted may be explained by behavior that evolved at lower prey availability in the lionfish's native range (Sih et al. 2004, Wolf and Weissing 2012). Foraging rates in Indo-Pacific lionfish aren't known, but the recent findings that their Indo-Pacific prey are naïve to their presence and that they hunt prey as effectively in the Indo-Pacific as the western Atlantic suggests that their foraging wouldn't be limited by prey availability, so why lionfish would have evolved such intensive defensive morphology and forage below the physiological limit I found is unclear (Cure et al. 2012, Lönnstedt and McCormick 2013, McCormick and Allan 2016). It's possible that lionfish have a different energetic physiology in their native range than what I observed in their invasive population, as thermal physiology has been found to differ between the two populations (Barker et al. 2017, Dabruzzi et al. 2017) and because invasive populations may express phenotypic traits that are selected against in their native range (Sakai et al. 2001, Davidson et al. 2011), but further research would be necessary to resolve these differences.

SDA_{total} was 26% lower at 32° than 26° C when divided by meal size to control for its effect, however whether this is due to the effect of temperature itself or its interactive effect with scope for activity is uncertain. Regardless, this indicates that lionfish have lower costs of digestion at temperatures approaching their thermal maximum. While the underlying mechanisms of SDA are not fully understood, a majority of it has been attributed to cellular-level protein handling, which may have been more efficient at 32° than 26° C. A thermal optimum for protein handling has been proposed as an explanation for temperature-dependent SDA in fish, which may relate to temperature dependency in growth, aerobic scope, and other physiological processes (Pannevis and Houlihan 1992, Eliason et al. 2008). Declining SDA_{total} with temperature has also been reported in Eurasian Minnow (*Phoxinus phoxinus*) and Atlantic Cod (*Gadus morhua*), which is believed to correspond to those species' respective temperature optima for growth (Cui and Wootton 1988, Garner et al. 1997, Tirsgaard et al. 2015). Temperature-specific growth rates have not been studied in lionfish to date, however they've been noted to grow faster nearer the equator (Edwards et al. 2014), so the decline in SDA_{total} I observed at 32° C may also indicate a high temperature optimum for growth. Lower SDA_{total} could also correspond to a lower apparent digestibility coefficient (ADC)—or the percentage of prey energy assimilated versus excreted—however ADC generally increases with temperature due to enhanced enzymatic activity in the gut, so my observed decline in SDA_{total} more likely reflects improved digestive efficiency (Hardewig and Van Dijk 2003, Kofuji et al. 2005). While this reduced digestion cost wasn't reflected in a lower $\mathrm{SDA_{coeff}}$ or shorter $\mathrm{SDA_{dur}}$ at 32° C—which may be due to variability imposed by single feeding, discussed below—it may largely benefit lionfish in warming ocean temperatures by freeing up energy for growth, reproduction, and dispersal.

$\mathrm{SDA_{peak}}$ was correspondingly lower at 32° than 26° C when divided by meal size to control for its effect, however it occupied the entirety of scope for activity in most fish at 32° C due to a 23% decrease in scope for activity from 26° C. So while higher temperatures give lionfish lower $\mathrm{SDA_{total}}$ they will simultaneously limit locomotory capacity during SDA—supporting a hypothesis by Norin and Clark (2017) that predicted as such—but as discussed above this is likely an evolved trait in lionfish that may not affect their performance or fitness (Steiner and Pfeiffer 2006). For example, a meal size of 5% BM would fully occupy scope for activity at 32° C but occupy 80% of scope for activity at 26° C, leaving 20% of scope for activity free for ecological energy demands (Fig. 5). Fu et al. (2008) also found peak SDA that can exceed maximum metabolic rate from exercise in Southern Catfish (*S. meridionalis*), but my findings are the first to show that the extent of this exceedance is temperature-dependent, which could have major fitness implications in species like *S. meridionalis* that lack defensive morphology and may need to escape predators during SDA. I also found that frequent feeding increased $\mathrm{SDA_{peak}}$ along with meal size, which likely reflects natural feeding in lionfish better than my single-feeding experiment. As such, based on Green et al.'s estimated daily foraging rate of 8.9% BM $\mathrm{day^{-1}}$, wild invasive lionfish likely exceed their scope for activity during $\mathrm{SDA_{peak}}$ frequently.

While scope for activity decreased between 26° and 32° C, I observed a higher range between $\mathrm{SDA_{peak}}$ and SMR in the 32° C treatment, suggesting absolute aerobic scope increased from 26° to 32° C. I intended to measure aerobic scope instead of scope for activity, but did not anticipate the extent to which SDA_{peak} exceeded MMR_{chase}, which in most fish species is the absolute maximum metabolic rate (Norin and Clark 2016). Many tropical and subtropical fish species typically live in temperatures at or near their thermal optimum for aerobic scope and will therefore experience declining performance and fitness with anticipated temperature increases from climate change (Nillson et al. 2009, Rummer et al. 2014). If aerobic scope does increase from 26° to 32° C in lionfish, lionfish would be robust metabolic performers in temperatures that are only a few degrees below their average thermal maximum of 35°-36° C, and possibly well-suited to cope with future temperature increases from climate change (Barker et al. 2017, Dabruzzi et al. 2017). This is contrary to the decline in aerobic scope predicted by the oxygen- and capacity-limited thermal tolerance hypothesis (OCLTT). Mounting evidence suggests the OCLTT hypothesis may not be as generalizable as once thought, and our results indicate that lionfish may be among the species that don't reflect its predictions (Norin et al. 2014, Jensen et al. 2017). Barker et al. (2017) and Dabruzzi et al. (2017) found temperature preferenda below this hypothetical ≥32° C temperature optimum for aerobic scope (28.7° and 23-24° C, respectively), however these were recorded in non-feeding lionfish, and fish may modulate their thermal preference to suit physiological processes with different temperature optima (Garner et al. 1998, Ward et al. 2010, Mohammed et al. 2016). Regardless, lionfish could hypothetically consume more prey at 32° C if their aerobic scope increases from 26° C, which is consistent with my observed 65% increase in average food consumption from 26° to 32° C, however further research to explicitly determine absolute aerobic scope is necessary to verify this hypothesis. Cerino et al. (2013) reported lower mean food consumption at 32° than 29° C but with considerable variability and less than half the sample size of my 32° C treatment, possibly confounding their results.

There was considerable individual variability in SDA_{total}, SDA_{dur}, SDA_{coeff}, and $SDA_{peak-hr}$ in the single-feeding experiment. Some of this variability may have been due to differences in caloric content among the silversides I fed my lionfish, but their more modest degree of variance suggests other factors were at play. Variable down-regulation of gut function during the 72-h fast before SDA was recorded may have been responsible. There was substantially less variability in SDA_{total} and SDA_{coeff} in the repeated-feeding experiment, possibly because the gut became up-regulated after the first feeding and behaved more consistently in subsequent meals. The time taken to down-regulate gut function varies widely among fish species, but in some like the Brown Surgeonfish (*Acanthurus nigrofuscus*) gut length can decrease by as much 46.7% in as little as 60 h (Montgomery and Pollak 1988, Zaldúa and Naya 2014). The timing and extent of gut regulation is driven by natural selection, and given that lionfish can consume large meals and survive for at least 3 months without feeding, they may be adapted to regulate their gut rapidly and widely (Fishelson 1997, Secor 2001, Zaldúa and Naya 2014, Côté and Smith 2018). Regardless of this hypothesis, the magnitude and cost of SDA was more consistent with repeated feeding. While individual feeding frequencies of wild lionfish aren't known, this consistency in cost has likely selected for frequent feeding in lionfish and concords with the high foraging rates observed in the invasive population (Côté and Maljković 2010, Green et al. 2011).

The greater consistency in SDA_{total} and SDA_{coeff} in the repeated-feeding experiment clarified several low-power relationships between metabolic phenotype and SDA from the single-feeding experiment. SMR and scope for activity varied almost twofold and threefold, respectively, but had no effect on either SDA_{total} or SDA_{coeff}. This differs from the findings of

Millidine et al. (2011), where juvenile Atlantic salmon (*S. salar*) with high SMR had costlier SDA but shorter SDA duration, which may allow for a higher food intake to support faster growth and agrees with the established link between higher-performing metabolic phenotypes and competitive ability that secures access to prey (Auer et al. 2015, Metcalfe et al. 2016). Intraspecific competition has not been studied in the lionfish's natural range, but their sedentary nature, high densities in the western Atlantic, generalist diet, and high strike success rates suggest that they likely don't compete for prey (Côté and Maljković 2010, Benkwitt 2015, Côté and Smith 2018), and so a high-performing metabolic phenotype would not be necessary to secure access to food. Lionfish were recently discovered to interact agonistically (Fogg and Faletti 2018), and while almost nothing is known about this behavior it may explain why I found metabolic phenotypes with a greater capacity for swimming performance in an otherwise sedentary species. Intraspecific variations in SMR are thought to give fish populations persistence under varying food availability, so my findings suggest this occurs independently of SDA in lionfish (Van Leeuwen et al. 2011, Norin and Clark 2016).

Metabolic phenotype affected SDA_{peak}, however, as SDA_{peak} increased with SMR as per Millidine et al. (2011), as well as with scope for activity. Millidine et al. (2011) proposed that peak SDA may correlate with growth rate as in other ectotherms (Vahl et al. 1984), however this has not been demonstrated in fish to date (Fu et al. 2008). Higher SDA_{peak} has also been proposed to represent enhanced digestive performance and efficiency (Brown and Cameron 1991, Leggatt et al. 2003, Fu et al. 2008), but I found no corresponding relationship between SDA_{coeff}, metabolic phenotype, and SDA_{peak} that would support this. Lionfish with high-SMR phenotypes may simply have higher SDA_{peak} due to a higher baseline metabolism from which SDA is measured in relation to. In other species this may pose a drawback due to SDA_{peak}

occupying more of aerobic scope (Norin and Clark 2017), but as discussed above this is unlikely to affect the performance or fitness of lionfish. As SDA_{dur} couldn't be calculated for multiple meals, the repeated-feeding experiment couldn't clarify the significant relationship between SDA_{dur} and scope for activity observed in the single-feeding experiment, but the lack of a scope for activity effect on SDA_{total} in both experiments suggests this may be a spurious relationship due to the aforementioned variability in the single-feeding experiment data.

Chapter 4. Conclusion and Future Directions

This study is the first to show how an invasive species' SDA and digestive physiology may enhance its invasiveness, supporting the supposition that species invasions can be explained in part by physiological characteristics (Lennox et al. 2015, Marras et al. 2015). The lionfish invasion is believed to be driven primarily by a lack of substantive natural predators and prey naivete (Côté and Smith 2018), but numerous other traits have been found that likely contribute to the lionfish's success, such as a capacity for rapid growth and reproduction (Morris et al. 2011, Albins and Hixon 2013), novel hunting techniques (Albins and Lyons 2012, McCormick and Allan 2016), parasite resistance (Tuttle et al. 2017), tolerance to extreme temperatures and salinities (Jud et al. 2015, Dabruzzi et al. 2017), and possibly heterosis (Wilcox et al. 2017). Given this complex range of invasion-facilitating traits, it's difficult to discern which among them are the most impactful. My findings add to this list of potential contributing factors, but by no means do they offer a sole explanation for the lionfish's success. Rather, they offer a mechanistic explanation for several of these factors—particularly feeding rate and temperature-dependent performance—and show a novel way that climate change could affect the lionfish invasion. Past studies have concluded that lionfish may benefit from warming ocean temperatures with an expanded range, shorter larval durations, and increased feeding rates (Kimball et al. 2004, Côté and Green 2012, Bernal et al. 2015). My findings agree with these studies and indicate a new way in which to understand the lionfish invasion in light of changing ocean temperatures.

Population and ecosystem models have used physiological characteristics and their relationship to environmental variables to predict how lionfish impact western Atlantic ecosystems, both currently and in the future, however some assumptions in these models are based on limited knowledge of lionfish physiology (Côté and Green 2012, Cerino et al. 2013, Chagaris et al. 2017). For example, I found an average SDA coefficient 30% lower than that assumed by Cerino et al. (2013) and subsequent models, as well as a higher prey consumption rate with increasing temperature, which may have led them to underestimate wild lionfish prey consumption and impact. The findings of this study and others like it may therefore improve such models. Further research is necessary, however, to determine whether thermal plasticity affects the magnitude of my observed temperature effect, as the response of metabolic rate to temperature may change with acclimation time to laboratory conditions (Sandblom et al. 2014, Auer et al. 2016). Longer acclimation times than what was possible in this study could confirm whether my findings are suitable for ecological and biogeographic modelling. Further research is also necessary to determine absolute aerobic scope in lionfish across temperatures and up to their thermal maximum—perhaps by force-feeding to illicit absolute MMR at SDA_{peak}— to establish a thermal performance curve that can better predict invasive lionfish performance in warming ocean temperatures (Jensen et al. 2017). Accurate models based on such physiological data could facilitate the control and management of the lionfish invasion by indicating where to spend limited funding and resources for maximum efficacy (Lennox et al. 2015, Chagaris et al. 2017).

This study also expands on understudied aspects of fish SDA, as well as methodology that may improve how SDA is studied. Despite a fair number of studies on SDA in fish (Secor 2009, Norin and Clark 2016), relatively few established the relationship between SDA and aerobic scope or other fitness-related physiological traits (Fu et al. 2008), and fewer yet have studied how these relationships change with temperature (Norin and Clark 2016). This speaks to the number of SDA studies conducted on aquaculture species, where studying the discrete cost of digestion can be useful in the absence of ecological constraints relevant to wild fish, which has left species in other taxonomic or ecological contexts underrepresented (Secor 2009). By studying a species very different in nature to most of those studied to date, I found a novel relationship between SDA, scope for activity, aerobic scope, and temperature, highlighting the value of studying SDA in more diverse species, as well as their response to ecologically-relevant environmental variables. SDA should ideally be studied in relation to as many biotic and abiotic variables as possible, but studying its response to temperature should be prioritized in particular, as it is highly influential to fish metabolism and increasingly relevant in predicting fish species' response to climate change (Fry 1971, Marras et al. 2015).

Th effect or repeated-feeding on SDA was examined in this study as well, whereas almost all SDA studies to date used only one feeding. While SDA_{total} did not differ with feeding frequency—as in those few past studies (Ross et al. 1992, Fu et al. 2005)—it had greater consistency in response to meal size and metabolic phenotype in fish fed repeatedly. As discussed in Chapter 3, this is likely due to consistency conferred from gut up-regulation and better approximates lionfish feeding in the wild (Green et al. 2011). Regardless of its exact cause, this suggests that feeding fish repeatedly—or at least at a regiment that better reflects a species' natural feeding frequency—is advisable to future SDA studies. Repeated-feeding does present some challenges to studying SDA, however, as it precludes a direct determination of an individual repeated meal's SDA_{total} and SDA_{coeff}. I attempted to predict an individual repeated meal's SDA_{total} based on its SDA integral up to the next feeding, using data from the single-feeding experiment, however the variability in the single-feeding data resulted in inaccuracies that precluded this method's use. As a result, it's unclear whether an individual repeated meal has a less costly SDA due to elevated gut metabolism from the previous meal, which I had originally hypothesized and intended to answer with this experiment. Future research may be able to answer this question by using a species with narrower gut regulation and a more consistent SDA response that allows for an accurate estimation of an individual repeated meal's SDA_{total}. Repeated feeding may not be possible in species that won't freely feed in respirometry chambers, however, highlighting the limitations of studying natural physiological processes in unnatural laboratory conditions.

Biologgers offer a compelling means to study physiological processes like SDA in wild fish, and without the potential confounds imposed by laboratory conditions (Butler et al. 2004, Cooke et al. 2016). I attempted to use surgically-implanted heart rate loggers (Star Oddi, Gardabaer, Iceland) to measure feeding and digestive physiology in wild lionfish (Fig. 7), as heart rate closely approximates metabolic rate and SDA in fish (Eliason et al. 2008). This would reveal the feeding frequency and approximate consumption rate of individual invasive lionfish in natural conditions, as well as their daily energy requirements and how much excess energy they can allot to growth and reproduction (Côté and Maljković 2010). While lionfish implanted with these loggers recovered and survived—allowing for the successful recording of heart rate—they became disinterested in feeding, and after numerous attempts I discontinued their use to instead focus on respirometry. Lucas et al. (1991) successfully recorded feeding in wild Northern Pike (*Esox Lucius*) with surgically-implanted heart rate loggers, and the same heart rate loggers I used have successfully recorded heart rate with no apparent duress in Sockeye Salmon (*Oncorhynchus nerka*) and three species of decapod crustaceans (McGaw et al. 2017, Prystay et al. 2017), suggesting some aspect of lionfish anatomy or physiology makes them ill-suited to these surgical implantations. The loggers had to be inserted posterior of the heart due to the pelvic fins being anteriorly displaced in lionfish, which may have applied pressure to the stomach or esophagus and dissuaded instrumented fish from eating. Smaller heart rate loggers may alleviate this issue and allow for the remote measurement of feeding and digestive physiology in wild lionfish, but at present the ones that I used are the smallest commercially available option. Though they couldn't measure SDA, other kinds of biologgers like electromyogram or acceleration sensors could provide valuable insight into lionfish feeding and behavior in the wild as well.

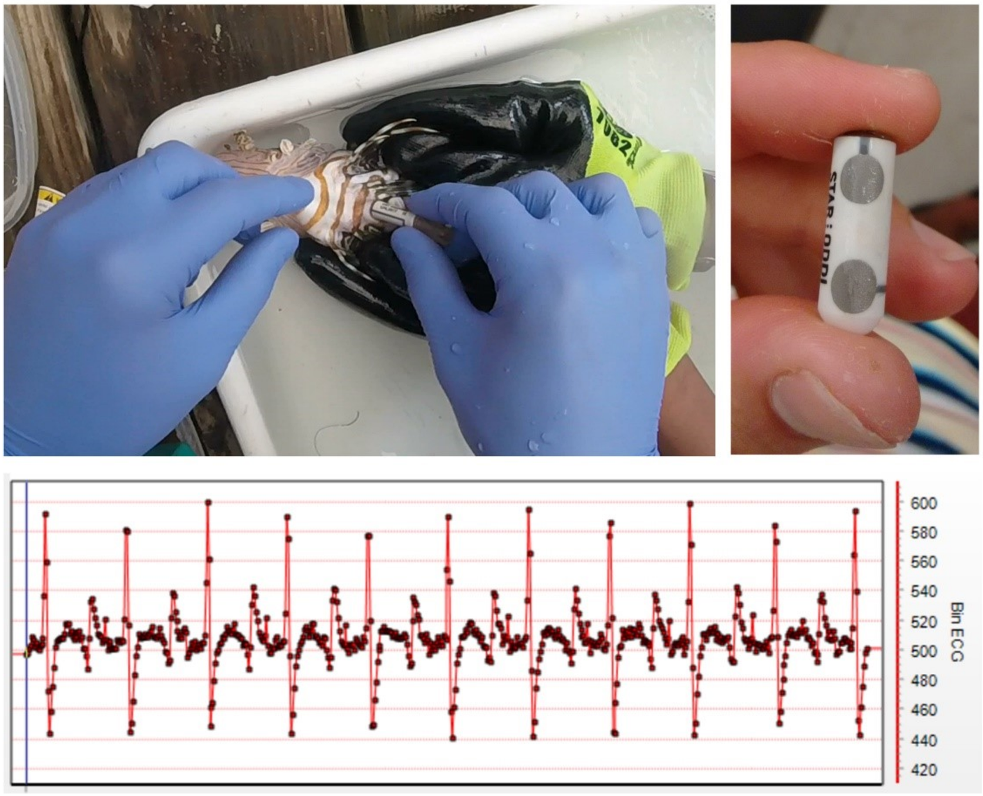

Figure 7. Example of a lionfish implanted with a Star-Oddi DST Micro-HRT heart rate logger (top images). The loggers are 25.4 mm in length, 8.3 mm in diameter, and weigh 3.3 g above water. Loggers were implanted ventrally so that the electrodes (dark circles in top right image) contacted the pericardium. Bottom image is a sample of heart rate recorded from a 300 g lionfish.

Supplementary Materials

Table 2. Results from a random-forest algorithm run on the single-feeding experiment data, with tree number set to 1,000. Mean square error increase indicates the relative importance of a predictor to a given SDA response variable. The variance explained by the model is the percentage of variance in the response explainable by all predictors in the random-forest.

SDA parameter	Predictor	Mean-square error increase (%)	Variance explained by model (%)
SDA$_{peak}$	Meal size	25.8%	66.2%
	Temperature	22.8%	
	SMR	18.5%	
	Scope for activity	3.7%	
SDA$_{total}$	Meal size	21.7%	11.1%
	Temperature	4.5%	
	Scope for activity	1.3%	
	SMR	-5.4%	
SDA$_{duration}$	Meal size	14.1%	-7.7%
	Temperature	3.7%	
	Scope for activity	2.2%	
	SMR	-7.6%	
SDA$_{peak\text{-}hr}$	Meal size	1.7%	-16.9%
	Scope for activity	0.6%	
	SMR	-0.3%	
	Temperature	-3.3%	
SDA$_{coeff}$	Scope for activity	3.4%	-17.7%
	Temperature	2.5%	
	SMR	-1.5%	
	Meal size	-4.3%	

Table 3. Linear models, their equations, and r^2 values for significant relationships in our results. Models for Peak SDA (SDA_{peak}) in both experiments and total SDA (SDA_{total}) in the repeated-feeding experiment include only predictors that were significant in their respective ANCOVA models. All other models for SDA parameters were selected with Akaike's Information Criteria (AIC) and have at least one significant predictor. Equations represent relationships between non-transformed data.

Feeding frequency	Response	Predictors	Temperature (C)	Model fit	Equation
Single	SDA_{peak}	Meal size, SMR	26°	$r^2_{adj}=0.85$	$SDA_{peak}=11.52\cdot\%BM+0.83\cdot SMR+97.35$
Single	SDA_{peak}	Meal size, SMR	32°	$r^2_{adj}=0.61$	$SDA_{peak}=11.12\cdot\%BM+0.83\cdot SMR+107.19$
Single	SDA_{total}	Meal size, scope for activity	26°	$r^2_{adj}=0.40$	$SDA_{total}=751.15\cdot\%BM+46.21\cdot scope-8008.93$
Single	SDA_{total}	Meal size, SMR	32°	$r^2=0.48$	$SDA_{total}=494.1\cdot\%BM-32.9\cdot SMR+5146.7$
Single	SDA_{coeff}	Scope for activity	26°	$r^2=0.33$	$SDA_{coeff}=0.01\cdot scope-0.837$
Single	SDA_{coeff}	Meal size	32°	$r^2=0.14$	$SDA_{coeff}=-0.79\cdot\%BM+17.07$
Single	SDA_{dur}	Meal size, scope for activity	26°	$r^2_{adj}=0.42$	$SDA_{dur}=4.86\cdot\%BM+0.797\cdot scope-103.32$
Single	SDA_{dur}	SDA_{total}, meal size, scope for activity	26°	$r^2_{adj}=0.94$	$SDA_{dur}=-0.0085\cdot SDA_{total}-1.51\cdot\%BM+0.405\cdot scope+35.4$
Single	SDA_{dur}	SDA_{total}, meal size	32°	$r^2_{adj}=0.51$	$SDA_{dur}=0.0095\cdot SDA_{total}-2.77\cdot\%BM+43.52$
Repeated	SDA_{peak}	Meal size, scope for activity, between-meal interval	26°	$r^2_{adj}=0.65$	$SDA_{peak}=22.06\cdot\%BM+0.76\cdot scope-0.9331\cdot interval+69.59$
Repeated	SDA_{total}	Meal size	26°	$r^2=0.79$	$SDA_{total}=707.8\cdot\%BM+1332.7$

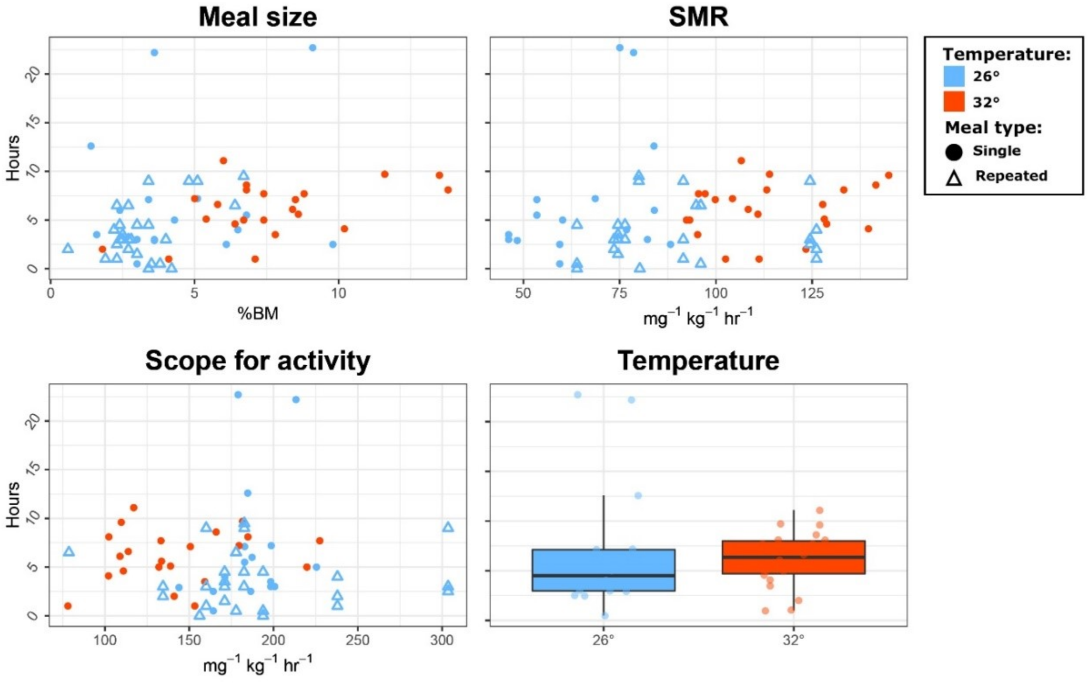

Figure 8. Relationships between specific dynamic action (SDA) time-to-peak ($SDA_{peak-hr}$), meal size, standard metabolic rate (SMR), scope for activity, and temperature (26° and 32 ° C). $SDA_{peak-hr}$ is the number of hours between feeding and peak SDA (SDA_{peak}). Meal size is measured as a percentage of body mass (%BM). Scope for activity is the difference between maximum metabolic rate at chase (MMR_{chase}) and SMR in an individual lionfish.

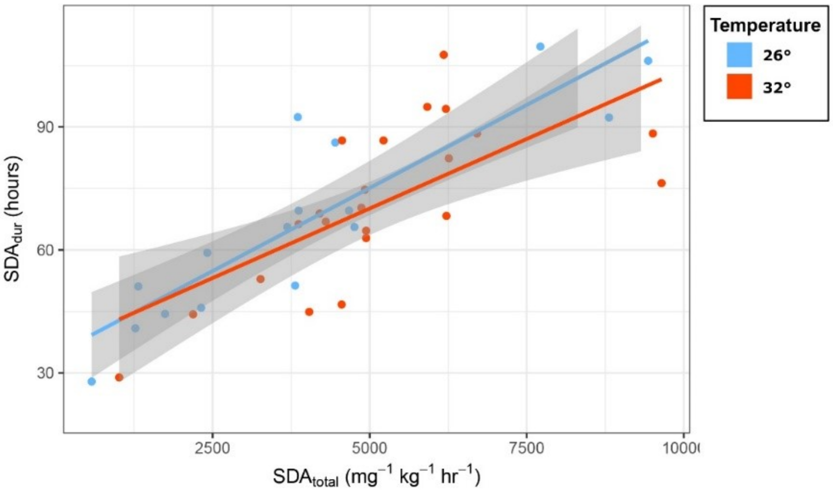

Figure 9. The relationship between specific dynamic action (SDA) duration (SDA$_{dur}$) and total SDA (SDA$_{total}$) as a function of temperature (26° and 32 ° C). Trendlines with 95% CI included for significant relationships.

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