

An appetite for invasion: digestive physiology, thermal performance, and food intake in lionfish

(Pterois spp.)

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Abstract

Species invasions threaten global biodiversity, and physiological characteristics may determine their impact. Specific dynamic action (SDA; the increase in metabolic rate associated with feeding and digestion) is one such characteristic, strongly influencing an animal's energy budget and feeding ecology. We investigated the relationship between SDA, scope for activity, metabolic phenotype, temperature, and feeding frequency in lionfish (*Pterois spp.*), an invasive species to western Atlantic marine ecosystems. Intermittent-flow respirometry was used to determine SDA, scope for activity, and metabolic phenotype at 26°C and 32°C. Maximum metabolic rate occurred during digestion, as opposed to exhaustive exercise as in more athletic species. SDA and its duration (SDA_{dur}) was 30% and 45% lower at 32°C than 26°C, respectively, and lionfish ate 42% more at 32°C. Despite a 32% decline in scope for activity from 26°C to 32°C, aerobic scope may have increased by 24%, as there was a higher range between standard metabolic rate (SMR) and peak SDA (the maximum postprandial metabolic rate). Individuals with high SMR and low scope for activity phenotypes had a less costly SDA and shorter SDA_{dur} but a higher SDA_{peak} . Feeding frequently had a lower and more consistent cost than consuming a single meal, but increased SDA_{peak} . These findings demonstrate that: 1) lionfish are robust physiological performers in terms of SDA and possibly aerobic scope at temperatures approaching their thermal maximum, 2) lionfish may consume more prey as oceans warm with climate change, and 3) metabolic phenotype and feeding frequency may be important mediators of feeding ecology in fish.

Introduction

Species invasions are a widely-recognized and growing threat to global biodiversity (Ricciardi et al. 2017). Dozens of fish species have become invasive worldwide as the result of human activity, and many more may become invasive with over 600 known introductions of non-native species (Gozlan 2008). The probability of an introduced species becoming invasive depends on ecological and life history traits (e.g. niche, growth rate, reproductive capacity, or tolerance to disturbance) which are underpinned by physiological characteristics (Van Kleunen et al. 2010, Kelley 2014, Lennox et al. 2015). Characteristics of metabolic rate—the rate at which an organism expends energy, commonly measured using oxygen consumption rate (MO_2)—are increasingly recognized as a potential determinant of invasion success (González-Ortegón et al. 2010, Maazouzi et al. 2011, Lejeusne et al. 2014, Lagos et al. 2017). Relatively few studies have examined their relevance to invasive fish, however, which is surprising given the number and impact of invasive fish species worldwide (but see Marras et al. 2015, McCallum et al. 2017, Behrens et al. 2017, Srean et al. 2017, Nati et al. 2018, Tessier et al. 2018).

A characteristic of metabolic rate that has largely been ignored in invasive species biology is specific dynamic action (SDA): the postprandial increase in metabolic rate associated with feeding

and digestion. Specific dynamic action represents the total energy expenditure of numerous pre-absorptive, absorptive, and post-absorptive pathways associated with feeding and digestion, and is typified by a rapid rise in metabolic rate up to a relatively short-lived peak (SDA_{peak}) followed by a longer decline (Jobling 1981, McCue 2006, Chabot et al. 2016; Fig. 1). Specific dynamic action is ecologically relevant because it can occupy a large proportion of a fish's energy budget in terms of its aerobic scope or scope for activity. Aerobic scope is the range between standard metabolic rate (SMR, the minimum metabolic rate required for maintenance) and maximum metabolic rate (MMR), whereas scope for activity is the range between SMR and active metabolic rate (AMR, the metabolic rate elicited from maximal exercise) (Sandblom et al. 2014, Norin and Clark 2016). Aerobic scope and scope for activity are equivalent and interchangeable in many species (i.e. $AMR=MMR$), however in some more sedentary species these characteristics are distinct as MMR may be achieved independently of exhaustive exercise (i.e. $AMR<MMR$) (Norin and Clark 2016). As SDA increases with meal size, a tradeoff occurs between feeding and maintaining a sufficient aerobic scope or scope for activity required to avoid predators, move through the environment, or find the next meal (Auer et al. 2015a, Norin and Clark 2017). This tradeoff may be mediated by the environment or physiological state (Secor 2009, Chabot et al. 2016, Metcalfe et al. 2016), but little work has investigated how this manifests in fish. This study explores three of these factors: 1) temperature, 2) metabolic phenotype, and 3) feeding frequency.

Temperature strongly influences both SDA and aerobic scope (Secor 2009, Farrell 2016). The effect of temperature on either aerobic scope or SDA has been described for many fish species, however the effect of temperature on these two traits together has been described for very few (but see Pang et al. 2010, Pang et al. 2011, Sandblom et al. 2014), none of which have been invasive species. Changes in aerobic scope or scope for activity have major implications to fish performance and fitness (Rummer et al. 2014, Farrell 2016, Norin and Clark 2016). Understanding the effect of temperature on SDA is necessary to contextualize the significance of these changes in aerobic scope, however, as aerobic scope and SDA can vary with temperature independently and in turn define the roles of feeding, exercise, and other energetic processes in a species' energy budget (Sandblom et al. 2014, Auer et al. 2015b, Metcalfe et al. 2016, Norin and Clark 2017). As such, the relationship between

SDA and temperature could play a significant role in the expected increase of ectothermic species invasions in aquatic ecosystems with climate change, particularly as temperature approaches species' thermal maxima where aerobic scope or scope for activity is often reduced (Côté and Green 2012, Marras et al. 2015).

In addition to temperature, metabolic phenotypes have been found to affect SDA in fish. Metabolic phenotypes are inherent individual variations (either genotypic or plastic) in SMR, routine metabolic rate (RMR, metabolic rate at regular activity levels), MMR, AMR, and aerobic scope or scope for activity, and are important determinants of a fish species' behavior, ecology, and life history (Cutts et al. 2002, Fu et al. 2008, Norin and Clark 2016). Millidine et al. (2009) found that juvenile Atlantic salmon (*Salmo salar*) with a high SMR phenotype had an energetically costlier SDA but a shorter SDA duration (SDA_{dur}) than those with a low SMR phenotype, which in salmonids is hypothesized as a tradeoff for faster growth. However, fish may express metabolic phenotypes in MMR, AMR, and aerobic scope independently of their SMR phenotype (Auer et al. 2015b, Metcalfe et al. 2016), the effects of which on SDA have not been explored to date.

Along with temperature and metabolic phenotype, the effect of feeding frequency on SDA and its occupation of aerobic scope or scope for activity has been relatively understudied in fish. Most SDA studies in fish have analyzed single meals (Secor 2009, Chabot et al. 2016), however many fish feed more frequently than the time required to fully digest a single meal, which in other ectotherms can lower the energetic cost of up-regulating gut function (Iglesias et al. 2003, Secor 2009, Zaldúa and Naya 2014). In turn, this may lower the cost of SDA relative to eating single, less frequent meals, however empirical support for this in fish remains sparse. Ross et al. (1992) and Guinea and Fernandez (1997) found no difference in the cost of SDA between one meal and several smaller, more frequent meals of the same total ration in Nile tilapia (*Oreochromis iuloticus*) and Gilt-headed sea bream (*Sparus aurata*), respectively, however frequent feeding caused a higher SDA_{peak} in these species, which has also been observed in frequently fed Southern catfish (*Silurus meridionalis*) (Fu et al. 2005). These studies were primarily concerned with fish aquaculture, and did not consider that such increases in SDA_{peak} may occupy a greater proportion of their aerobic scope or scope for activity. Such a tradeoff may mediate feeding rates in species that must maintain an adequate aerobic scope or

scope for activity for predator avoidance or other forms of exercise during SDA (Norin and Clark 2017), but this has yet to be explored experimentally.

We sought to explore these paradigms in invasive lionfish (*Pterois spp.*). Lionfish are mid-sized demersal predators native to the Indian and Pacific oceans that have been invasive to the western Atlantic Ocean since 2001 and the Mediterranean since 2012 (Hixon et al. 2016, Bariche et al. 2017). Their phylogeny has recently been contested (Wilcox et al. 2017), so lionfish will be referred to generically henceforth. Their invasion is driven mainly by a lack of natural predators, prey naivety, and unique hunting traits not found in native predators, which allows lionfish to spread and forage with few, if any, limits (Valdivia et al. 2014, McCormick and Allan 2016, Green et al. 2019). Lionfish threaten western Atlantic marine ecosystems by consuming small-bodied fish and crustaceans, significantly lowering their populations and even extirpating them locally (Benkwitt 2015, Palmer et al. 2016, Ingeman 2016, South et al. 2017), facilitating coral-smothering algal growth by removing grazers (Kindinger and Albins 2017), and outcompeting native mesopredators (Raymond et al. 2015, Curtis et al. 2017). Green et al. (2011) estimated an adult lionfish could consume 8.9% of its body mass per day based on field observations in The Bahamas, which was more than twice their required daily energy intake for maintenance (i.e. SMR) as estimated by Côté and Maljković (2010). This excess energy assimilation allows lionfish to grow and reproduce much faster than native mesopredators, reaching sexual maturity in less than a year and spawning as frequently as every 4 days (Morris 2009, Côté et al. 2013).

A broad thermal tolerance spanning 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 voluntary food intake found by Cerino et al. (2013). Dabruzzi et al. (2017) found a lower temperature preferendum between 23°C and 24°C in Indo-Pacific lionfish, however they found a similar mean critical thermal minimum ($CT_{min}=12.1^{\circ}$) and maximum ($CT_{max}=35.3^{\circ}C$) to that of Barker et al. (2017) ($CT_{min}=12.1^{\circ}C$ and $CT_{max}=36.5^{\circ}C$). Invasive lionfish have therefore been found to have a preferendum and food intake optimum close to their upper thermal threshold, a typical trait in tropical fish (Norin et al. 2014,

Rummer et al. 2014), however performance-based physiological metrics such as aerobic scope or scope for activity have not been tested in lionfish across temperatures to date. 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 have been predicted to increase feeding rates in invasive lionfish (Côté and Green 2012, Cerino et al. 2013), however feeding metabolism (i.e. SDA) and its relationship to temperature has not been studied in lionfish to date.

The objective of the study was to determine the effect of temperature (26°C vs. 32°C), metabolic phenotype (SMR and scope for activity) and feeding frequency (single vs. repeated feeding) on SDA in invasive lionfish and whether these relationships may facilitate the invasiveness of this species as oceans warm with climate change. 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). We hypothesized that 1) SDA and SDA_{dur} would decrease with temperature, given that lionfish optimally feed close to their upper temperature thresholds, while SDA_{peak} would increase due to the higher metabolic demands of increased temperature, 2) lionfish with higher SMR and larger scope for activity would have a greater SDA but shorter SDA_{dur} as per the findings of Millidine et al. (2009), 3) As temperatures increase, scope for activity would decrease and SDA would occupy a greater proportion of it in turn 4) The SDA of frequent feeding would be lower than that of a single feeding, 5) SDA_{peak} of a repeated meal would be higher when the previous meal was larger and more recent, and 6) Lionfish would eat larger repeated meals when residual metabolic rate from the previous meal's SDA was lower and occupying less of its scope for activity, and when the previous meal was smaller and less recent.

Methods

Animal collection and husbandry

Lionfish were collected on SCUBA with plastic hand nets from patch reefs in the Bight of Rock Sound (24° 50' 28 N, 76° 17' 13 W) in the winter (Jan-Mar) and summer (Jun-Jul) of 2017 and winter of 2018 (Jan-Feb) ($\bar{x} = 135.5 \pm 9.5$ g body mass (BM); all data are reported as mean \pm standard error; sex unidentifiable as $GSI < 1\%$ BM in all individuals). All collections were from less than 4 meters depth to prevent barotrauma. Collected lionfish were transported to the Cape Eleuthera Institute wet lab and held in outdoor circular 750 liter tanks with no more than ten individuals per tank that were aerated and continuously supplied with fresh seawater (5 L min^{-1}) at ambient temperature (see below). All lionfish were acclimated to lab conditions for a minimum of 5 days and held for a maximum of 22 days before metabolic rate measurements, and were fed live silversides (*Atherinomorus stipes*) to satiation (when feeding ceased after the addition of new prey) every 3 to 5 days. Live silversides were used as prey because the lionfish would not eat dead prey, a typical behavior of the species (Cerino et al. 2013, Hixon et al. 2016).

Respirometry

An 8-chambered intermittent-flow respirometry system (Loligo Systems, Viborg, Denmark) was used to measure oxygen uptake rates (MO_2) in individual lionfish. 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, Deizisau, Germany). The chamber lids had 3 cm ports sealed with rubber stoppers through which prey fish could be fed *in situ* and with minimal disturbance during measurements. Chambers were immersed in 2 570 L raceways (300 x 30 x 60 cm) supplied with filtered and aerated seawater. Loligo mini sensor oxygen probes (Witrox, Loligo Systems, Viborg, Denmark) were calibrated before each measurement period to 0% and 100% air saturation using a seawater-sodium sulphite solution and air-saturated seawater, respectively. Oxygen consumption (MO_2) was recorded in a closed loop mixed by a recirculating pump for 10-min, preceded by a 19-min flush period to restore oxygen saturation levels and 1-min wait period. 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°C (n = 13) and 32°C (n = 16), and a feeding frequency experiment was conducted at 26°C (n = 13). The feeding frequency experiment was planned to include a 32°C treatment, but unfortunately could not be completed due to a limited timeframe. 26°C was chosen as it represents contemporary ambient winter sea 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 prey consumption rate at 32°C. In the single feeding experiment, the 26°C treatment was performed in Mar-2017 and Jun-2017, and the 32°C treatment was performed in Jul-2017. The feeding frequency experiment was conducted in Jan-2018 and Feb-2018. There was no significant difference in body mass or SMR between the 26°C treatment measurement in Mar-2017, the 26°C treatment measurement in Jun-2017, and the repeated feeding treatment measured in Jan-2018 and Feb-2018 at 26°C according to Welch's t-tests, and there was no consistent temporal change in SMR or scope for activity between measurement rounds in any treatment, suggesting minimal possibility for an acclimation effect (Sandblom et al. 2014). All lionfish were used once.

In both experiments, lionfish were fed to satiation in their holding tanks and then fasted for 48 hr prior to respirometry to ensure a post-absorptive state, which was confirmed by a plateaued MO_2 trace as SMR was measured (see below). Lionfish were transferred directly from their holding tank into respirometry chambers and MO_2 was recorded for 24 hr to calculate SMR. The difference in temperature between the holding tanks and the respirometry chambers was $\pm 2^\circ C$ or less, typical of diel variation in the shallow patch reefs from which the lionfish were collected. Lionfish were then fed live silversides directly in their chambers during crepuscular hours, and MO_2 was immediately recorded to calculate SDA. Because lionfish would not consistently eat the meal sizes we sought to measure, we decided to feed each fish to satiation in order to capture a range of meal sizes that could be measured as a continuous variable. Meal sizes were measured by weighing individual silversides

prior to putting them in the respirometry chambers. One silverside was presented to the lionfish at a time and were sequentially added until the lionfish ceased feeding. In the single feeding experiment, SDA was recorded over 96 hr following a single feeding that occurred between 17:00 and 19:00. In the feeding frequency experiment, SDA was recorded for 72 hr while lionfish were fed to satiation every morning between 06:00 and 08:00 and every evening between 17:00 and 19:00, followed by 60 hr without feeding. Rations ranged between 0.6% and 13.8% body mass (1.0-20.4 g). AMR was determined at the end of each trial by chasing each lionfish 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 lionfish were then immediately returned to their respirometry chambers and MO_2 was recorded.

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.

Prey calorimetry

The gross energy content of 10 silversides collected in winter 2017 were determined using an Oxygen bomb calorimeter (Parr Instrument Co., Moline, USA). Average gross energy density was 13.79 ± 0.87 kJ g^{-1} (wet weight), similar to the energy density of demersal western Atlantic reef fish that lionfish would prey upon (Schwartzkopf and Cowan 2017, Welicky et al. 2018). Pettitt-Wade et al. (2011) found no difference in the energy density of small forage fish including *A. stipes* between seasons in The Bahamas, so this energy density was used for all treatments and experiments.

Data analysis

Raw MO_2 was corrected for microbial background respiration for each chamber in each trial. 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 the slope of each MO_2 measurement 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 lionfish 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 hr) to account for short bouts of activity in some fish per Eliason et al. (2007). Active metabolic rate (AMR) and SDA_{peak} were derived from unblocked MO_2 . Standard metabolic rate (SMR), RMR, and SDA were derived from MO_2 block minimums. Standard metabolic rate (SMR) was calculated as the average of the lowest 10th percentile of MO_2 recorded over 24 hr before feeding and following a 48 hr fasting period. RMR was calculated as the average MO_2 of the 18 hr before feeding after a 6 hr recovery period following placement in the respirometer. Specific dynamic action duration (SDA_{dur}) was calculated as the number of hours between feeding and the third point of postprandial MO_2 to fall below RMR. Four lionfish in the single feeding experiment and 4 lionfish in the feeding frequency experiment had postprandial MO_2 that did not return below RMR within 96 hr but were within 20% of it and trending downward, for which SDA_{dur} was extrapolated to the slope of the last period of declining MO_2 derived from a 5th-order polynomial trendline fitted to the SDA response. Specific dynamic action (SDA) was calculated by integrating the area under the curve of postprandial MO_2 over SDA_{dur} minus SMR (Fig. 1A). Routine metabolic rate (RMR) was chosen as the endpoint of SDA_{dur} because many lionfish had postprandial MO_2 that did not return to SMR before the end of the experiment, but was not used as the baseline from which SDA is measured because it was consistently higher than SMR and therefore a less accurate measure of true resting metabolic rate. SDA_{peak} exceeded AMR in many lionfish and precluded a reliable measurement of aerobic scope, so scope for activity was used instead and calculated as the difference between AMR and SMR. The cost of SDA as a percentage of energy consumed is termed the SDA coefficient ($\text{SDA}_{\text{coeff}}$). This was calculated with the equation $\text{SDA}_{\text{coeff}} = (E_{\text{SDA}} / E_{\text{meal}}) * 100$, where E_{SDA} is the energy spent on SDA assuming 1 g of O_2 is associated with the release of 13.6 kJ of energy (Cho et al. 1982) as the rate of sequential fuel use (Ferreira et al. 2019) is not known for lionfish, 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).

In the feeding frequency experiment, single values for SDA, meal size, and SDA_{coeff} across repeated feedings were determined by integrating all repeated meals, summing repeated meal sizes, and using these values in the equation above, respectively (Fig. 1B).

Body mass (BM) varied 7.6-fold in the single feeding experiment (43.5-331.5 g, $\bar{x} = 140 \pm 11.3$ g) and 3.5-fold in the feeding frequency experiment (44-155 g, $\bar{x} = 92.3 \pm 9.9$ g). Log10-transformed whole-animal SMR ($\text{mg O}_2 \text{ hr}^{-1}$) varied allometrically when regressed against log10-transformed BM (kg) at both temperatures used in this study. Oxygen consumption (MO_2) was therefore mass-adjusted to that of a 140 g lionfish using the equation $y_{0.14 \text{ kg}} = y_M (M \cdot 0.14^{-1})^{(1-b)}$, where $y_{0.14 \text{ kg}}$ is MO_2 mass-adjusted to a 140 g lionfish, y_M is oxygen consumption rate (MO_2) of a lionfish 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 ($b = 0.835$). Results are presented using mass-adjusted data.

Statistical analysis

Separate ANCOVA models were fit for each SDA parameter (SDA , SDA_{coeff} , SDA_{peak} , and SDA_{dur}) to test the effect of temperature, meal size, SMR, and scope for activity. All but one of the parameters were normally distributed, homoscedastic, and met assumptions of ANCOVA, with SDA_{coeff} log10-transformed to meet assumptions of normality and homoscedasticity. Interactions were determined by including interaction terms between temperature and each covariate in the ANCOVA model. Relationships between the predictors were also analyzed, using Welch's t-tests or regression as appropriate. Significance was determined with P -values in all models. All statistical tests were performed in Rstudio (Rstudio Inc, Boston, USA).

Data from the feeding frequency experiment were analyzed in 3 separate ways, each testing the effect of meal size, SMR, and scope for activity as in the single feeding experiment. The first used multiple regression to determine whether food intake 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} . Variance inflation factors were below 3. Akaike information criteria was used to determine the optimal combination of predictors for each linear model. The third used ANCOVA models to test the effect of frequent versus single feeding on SDA and SDA_{coeff} while controlling for meal size, SMR, and scope for activity as covariates.

Results

1. Single feeding experiment

1.1 Relationships between predictors: temperature, meal size, SMR, and scope for activity

Lionfish ate significantly more ($P = 0.003$) in the 32°C treatment than the 26°C treatment, with a 42% increase in prey consumption from 26°C ($\bar{x} = 4.5 \pm 0.59\%$ BM, 1.4-9.1% BM) to 32°C ($\bar{x} = 7.8 \pm 0.81\%$ BM, 1.8-13.8% BM). The lionfish expressed a range of metabolic phenotypes, with a two-fold variation in both SMR and scope for activity in either temperature treatment. Scope for activity did not significantly vary with SMR, but rather significantly increased with AMR ($P < 0.01$). Food intake did not significantly vary with SMR or scope for activity.

SMR significantly differed between temperatures ($P < 0.001$), with a 59% increase from the 26°C treatment ($\bar{x} = 69.6 \pm 4.0$ mg kg⁻¹ hr⁻¹,) to the 32°C treatment ($\bar{x} = 118.4 \pm 3.9$ mg kg⁻¹ hr⁻¹), while AMR did not significantly differ between the 26°C treatment ($\bar{x} = 257.0 \pm 7.8$ mg kg⁻¹ hr⁻¹) and the 32°C treatment ($\bar{x} = 245.9 \pm 7.8$ mg kg⁻¹ hr⁻¹) (Fig 2). SDA_{peak} exceeded AMR in many lionfish and precluded a reliable measurement of true aerobic scope (MMR-SMR), so scope for activity (AMR-SMR) was used instead. Scope for activity significantly differed between temperatures ($P < 0.001$), declining 32% from the 26°C treatment ($\bar{x} = 187.4 \pm 5.8$ mg kg⁻¹ hr⁻¹) to the 32°C treatment ($\bar{x} = 127.5 \pm 6.6$ mg kg⁻¹ hr⁻¹) (Fig. 2). Aerobic scope may have increased from 26°C to 32°C, however, as the range between SMR and SDA_{peak} from the largest meals at these respective temperatures increased by 24% (26°C: $SDA_{peak}=193.7$ mg kg⁻¹ hr⁻¹ for a 9.1% BM meal; 32°C: $SDA_{peak}=262.6$ mg kg⁻¹ hr⁻¹ for a 13.8% BM meal).

1.2 Effects of temperature, meal size, SMR, and scope for activity on SDA parameters

The effect of temperature on SDA approached significance ($P = 0.08$). When divided by meal size to control for its positive effect ($P < 0.001$), SDA was 29.6% lower at 32°C ($\bar{x} = 685.2 \pm 51.2 \text{ mg kg}^{-1} \text{ hr}^{-1} \% \text{BM}^{-1}$) than 26°C ($\bar{x} = 972.8 \pm 130.7 \text{ mg kg}^{-1} \text{ hr}^{-1} \% \text{BM}^{-1}$) (Fig. 3A, 3D). $\text{SDA}_{\text{coeff}}$ was $15.2 \pm 1.6\%$ and did not significantly vary with meal size (Fig 3E). $\text{SDA}_{\text{coeff}}$ did not significantly differ between temperatures, but was 7% lower at 32°C ($\bar{x} = 15.9 \pm 2.7\%$) than 26°C ($\bar{x} = 14.7 \pm 2\%$) (Fig 3H)

SDA significantly varied with SMR ($P = 0.02$) and scope for activity ($P = 0.01$), decreasing with SMR and increasing with scope for activity when divided by meal size to control for its effect (Fig. 3B, 3C). Model results suggest SDA can be up to 33% lower at the highest SMR we observed at a given temperature versus the lowest, and up to 46% lower at the lowest scope for activity versus the highest (Table S1).

SDA_{peak} significantly differed between 26°C and 32°C ($P < 0.001$). When divided by meal size to control for its positive effect ($P < 0.001$), SDA_{peak} was 30% lower at 32°C than 26°C (Figs 3I, 3L). SDA_{peak} significantly increased with SMR ($P = 0.005$) and approached significantly increasing with scope for activity ($P = 0.057$) (Figs 3J, 3K). SDA_{peak} occupied the entirety of scope for activity (i.e. $\text{SDA}_{\text{peak}} > \text{AMR}$) in 88% of the feedings at 32°C, versus 23% at 26°C. All meal sizes created an SDA_{peak} that occupied more than 64% of a fish's scope for activity. Model results for SDA_{peak} suggests meal sizes of 8.4% and 4.2% BM would occupy the entirety of scope for activity at 26°C and 32°C, respectively, (i.e. $\text{SDA}_{\text{peak}} = \text{AMR}$) for fish with average SMR and scope for activity at those respective temperatures (Fig. 2; Table S1). The average time taken to reach SDA_{peak} after feeding was $6.6 \pm 1 \text{ hr}$.

The effect of temperature on SDA_{dur} approached significance ($P = 0.053$). When divided by meal size to control for its positive effect ($P = 0.05$), SDA_{dur} was 45% lower at 32°C ($\bar{x} = 10.1 \pm 1 \text{ hr \%BM}^{-1}$) than 26°C ($\bar{x} = 18.2 \pm 2.4 \text{ hr \%BM}^{-1}$) (Fig 3M, 3P). SDA_{dur} ranged from 27.9 to 109.6 h, significantly increased with scope for activity ($P = 0.02$), and approached significantly decreasing with SMR ($P = 0.09$) (Fig 3N, 3O). For a 5% BM meal, for example, our model results suggest that

SDA_{dur} would be up to 23.8 hr shorter at the lowest scope for activity we observed at a given temperature versus the highest, and up to 7.7 hr shorter at the highest SMR versus the lowest (Table S1). While SDA_{dur} for some larger meals exceeded the post-feeding 48-72 hr in which SMR was measured, MO₂ had stabilized during this window in all lionfish and did not steadily decline as in an absorptive state, suggesting the measurement of SMR was not confounded by elevated post-feeding metabolism from the satiation feeding prior to measurement.

2. Feeding frequency experiment

2.1 Effects of previous meals on feed intake and SDA_{peak}

Twenty-seven repeated meals were recorded for 13 lionfish, which ate between 2 and 5 meals each. Nine of these were eaten within 12 hr of the previous meal, 8 were eaten within 24 h, and 10 were eaten within 36 to 60 h. Food intake of repeated meals ranged from 0.6% to 8.5% BM ($\bar{x} = 3.7 \pm 0.3\%$) and varied among meals for individual lionfish. As in the single feeding experiment, individuals varied in metabolic phenotype, with a 1.5-fold variation in SMR and a two-fold variation in scope for activity. Food intake did not significantly vary with SMR or scope for activity.

Food intake had no relationship to how much of an individual's scope for activity was being occupied by residual SDA from the previous meal at the time of feeding (Fig 4D), nor the previous meal's size, SDA integral, or the interval between meals (Fig 4A-4C). SDA_{peak} of repeated meals significantly increased with meal size ($P < 0.001$), shorter intervals between meals ($P = 0.02$), and larger scope for activity ($P < 0.001$), and was not significantly affected by SMR, previous meal size, or the SDA integral of the previous meal (4E-4G).

2.2 Frequent feeding versus single feeding

Feeding frequency significantly affected SDA ($P < 0.001$) and was 15% lower for frequent feeding ($\bar{x} = 823.5 \pm 34.2 \text{ mg kg}^{-1} \text{ hr}^{-1} \% \text{BM}^{-1}$) than for one feeding ($\bar{x} = 972.8 \pm 130.7 \text{ mg kg}^{-1} \text{ hr}^{-1} \% \text{BM}^{-1}$) when divided by meal size to control for its positive effect ($P < 0.001$) (Fig 4H). There was also higher variability of SDA in the single feeding treatment ($\sigma_{\bar{x}} = 3,150 \text{ mg kg}^{-1} \text{ hr}^{-1}$) versus the repeated feeding treatment ($\sigma_{\bar{x}} = 2,641 \text{ mg kg}^{-1} \text{ hr}^{-1}$) (Fig 4H). Consistent with the single feeding experiment, SDA significantly varied with SMR ($P = 0.01$) and approached significantly varying with scope for activity ($P = 0.06$), decreasing with SMR and increasing with scope for activity when

divided by meal size to control for its effect (Fig 3A-3C). SDA_{coeff} across repeated meals was not significantly affected by meal size (Figs 3E-3G). Heterogeneity of variance in SDA_{coeff} between single and repeated meals precluded the use of an ANCOVA model, so it was assessed qualitatively. SDA_{coeff} of repeated meals ($\bar{x} = 10.1 \pm 0.34\%$) was 36% lower than that of single meals ($\bar{x} = 15.8 \pm 2.7\%$), and SDA_{coeff} of single meals had much higher variability ($\sigma_{\bar{x}} = 9.7\%$) than that of repeated meals ($\sigma_{\bar{x}} = 1.1\%$) (Fig 4I).

Discussion

We found several lines of evidence that lionfish have physiological traits that could facilitate their invasiveness, and that they may be robust to warming temperatures from climate change due to both their energetic and digestive physiologies. These can be broadly categorized into: 1) the relationship between SDA, energetic physiology, and feeding behavior 2) the effects of temperature 3) the effects of metabolic phenotype, and 4) the effects of feeding frequency.

1) SDA, energetic physiology, and feeding behavior

We found that lionfish have a physiology that prioritizes feeding over movement to a greater extent than that described in most other fish species to date. Maximum metabolic rate (MMR) is attained during exhaustive exercise in most fish species and not during digestion (Norin and Clark 2016). However, in this study, we observed lionfish routinely attain higher metabolic rates during SDA following voluntary feeding than from exhaustive exercise, with SDA_{peak} exceeding AMR by as much as 1.7 times for large meals at 32°C. 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, whether its scope for activity was exceeded, or how large or recent that previous meal was. In addition, the SDA we observed of even the smallest meals ($< 2.5\%$ BM) occupied at least 64% of scope for activity at 26°C and at least 80% at 32°C. Together, these findings suggest that the lionfish's defensive venomous spines may allow them to feed without a clear metabolic tradeoff to the locomotory capacity that most other species need to preserve to avoid predators or continue moving during digestion (Millidine et al. 2009, Norin and Clark 2017). Lionfish may retain some locomotory capacity during SDA despite exceeding the AMR we recorded, as AMR has been shown to increase in fed versus unfed fish up to 14% in European seabass (*Dicentrarchus*

labrax), 23% in Common carp (*Cyprinus carpio*), and 26% in Southern catfish (*S. meridionalis*) (Dupont-Prinet et al. 2009, Jourdan-Pineau et al. 2010, Pang et al. 2010, Zhang et al. 2012), however feeding has been found to have no effect on AMR in rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*Oncorhynchus tshawytscha*), goldfish (*Carassius auratus*), and qinbo (*Spinibarbus sinensis*) (Alsop and Wood 1997, Thorarensen and Farrell 2006, Zhang et al. 2012). As AMR was measured in fasted lionfish, we may be underestimating the meal sizes at which SDA fully occupies their scope for activity, however their scope for activity would likely still be very small or nonexistent when digesting medium or large meals. Regardless, this lack of a clear metabolic tradeoff between digestion and locomotory capacity would underpin the lionfish's high feeding rates and ability to assimilate excess energy for the rapid growth, reproduction, and dispersal that fuels their invasion (Côté and Maljković 2010, Green et al. 2011, Côté et al. 2013), and may be considered one of several traits that have made lionfish such a successful and impactful invader.

Furthermore, the readiness at which lionfish eat meals that exceed their scope for activity seems to be driven by the ability to eat exceptionally large meals, as opposed to an exceptionally low AMR or high SMR. Lionfish exhibited an SMR and AMR comparable to that of another coral reef predator at a similar temperature, Leopard coral grouper (*Plectropomus leopardus*) (Messmer et al. 2017), as well as a decline in factorial aerobic scope (the proportional difference between SMR and MMR or AMR) with increasing temperature almost identical to that of another sedentary predator, Shorthorn sculpin (*Myoxocephalus scorpius*) (Sandblom et al. 2014). As such, lionfish appear to readily exceed their scope for activity during SDA because they eat very large meals, suggesting this phenomenon could occur in other species with a similar scope for activity that also eat large meals. For example, Sandblom et al. (2014) found that a 5% BM meal occupied most of the scope for activity in *M. scorpius*, however this species has been observed to voluntarily eat meals up to 12.7% BM at a similar temperature (Johnston and Battram 1993), suggesting SDA in *M. scorpius* may exceed scope for activity to a similar extent as what we observed in lionfish.

As far as we are aware, this is the first study to experimentally test the role of the tradeoff between SDA and aerobic scope or scope for activity in a fish species' feeding behavior and food intake. Contrary to what we hypothesized, lionfish did not mediate their food intake based on the

occupation of SDA in their scope for activity or its exceedance of AMR. Lionfish foraging may instead be constrained by their MMR, equivalent to the SDA_{peak} of the largest possible consumable meal. The largest observed meal in this study may have approached this at 13.8% BM, which produced an SDA_{peak} 1.7 times greater than that fish's scope for activity. Observations of wild lionfish suggest they forage below such a hypothetical limit, however. Green et al. (2011) observed an 8.9% BM day⁻¹ consumption rate at 26°C in wild lionfish in The Bahamas, which was spread across smaller meals during morning and evening crepuscular hours. Based on our model results, a single 8.9% BM meal would produce an SDA_{peak} only marginally above the average AMR we observed at 26°C, and far below a hypothetical MMR from maximal feeding (Fig. 2). As such, a higher foraging rate would be expected in wild lionfish, suggesting that factors other than metabolic constraints mediate their foraging. Identifying these would be necessary to fully understand how climate change and a changing ocean environment are affecting the lionfish invasion (Côté and Smith 2018, Green et al. 2019).

2) Temperature

Our results showed that invasive lionfish digest meals more efficiently and rapidly at 32°C than 26°C, suggesting they may benefit from some ocean warming by virtue of their digestive physiology. SDA was 29.6% lower at 32°C than 26°C when divided by meal size to correct for its effect. This decline in SDA was reflected in a 7% decrease in SDA_{coeff} and a 45% decrease in SDA_{dur} as well. In addition, SDA_{coeff} did not differ with meal size, suggesting the decrease in SDA, SDA_{coeff} , and SDA_{dur} was not due to the increased food intake in the 32°C treatment. 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°C than 26°C. A thermal optimum for protein handling has been proposed as an explanation for temperature-dependent SDA in fish, which may underpin temperature dependency in growth, aerobic scope, and other physiological processes (Pannevis and Houlihan 1992, Eliason et al. 2008, Tirsgaard et al. 2015). Lower SDA could also correspond to a lower apparent digestibility coefficient (ADC; or the percentage of prey energy assimilated versus excreted), however ADC increases with temperature due to enhanced enzymatic activity in the gut, suggesting our observed decline in SDA reflects improved digestive efficiency

(Hardewig and Van Dijk 2003, Kofuji et al. 2005). Altogether, the reduced cost and duration of digestion observed in this study will likely benefit lionfish in warming ocean temperatures by allowing them to feed more frequently and allow more energy for growth, reproduction, and dispersal. Such a decrease in SDA at high temperatures may not necessarily be unique to lionfish, however, as research on warm water fish species' SDA near their upper thermal limits remains sparse. Nevertheless, invasive lionfish may stand to benefit from such a decrease in SDA more than native species, given their advantages of having naïve prey, a lack of natural predators, and unique hunting traits (Côté and Smith 2018, Green et al. 2019) and our aforementioned finding that they prioritize feeding over movement and predator avoidance to a greater extent than that described in any other species studied to date (Norin and Clark 2016).

Relative SDA_{peak} was correspondingly lower at 32°C than 26°C (that is, SDA_{peak} 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 32% decrease in scope for activity from 26°C. So while higher temperatures give lionfish lower SDA they will simultaneously limit locomotory capacity during digestion. For example, a meal size of 4.2% BM would fully occupy the scope for activity of an average lionfish at 32°C but leave 21% of its scope for activity free at 26°C for other energetic processes (Fig. 2), a similar trend observed by Sandblom et al. (2014) in Shorthorn sculpin (*M. scorpius*). This tradeoff is less likely to affect lionfish performance or fitness compared to other species (Norin and Clark 2017), as we found that they feed independently of how much their scope for activity is occupied by their previous meal, as discussed above.

While scope for activity decreased between 26°C and 32°C, we observed a higher range between SMR and SDA_{peak} of the largest meal at 32°C, suggesting aerobic scope increased from 26°C to 32°C. If so, lionfish would be robust performers in temperatures that are only a few degrees below their previously reported average thermal maximum of 35-36°C (Barker et al. 2017, Dabruzzi et al. 2017). They could hypothetically consume more prey at temperature increases projected for their invaded range in this case (Bernal et al. 2015), consistent with our observed 42% increase in average food intake from 26°C to 32°C. Cerino et al. (2013) reported the opposite in invasive lionfish, with a decrease in food intake between 29°C and 32°C, however they used only half the sample size of our

32°C treatment and reported considerable variability in it as well. This suggests that prey consumption increases up to higher temperatures than was previously thought in lionfish, and that subsequent ecosystem models based on the food intake reported in Cerino et al. (2013) may be an underestimation of the impact invasive lionfish have at these temperatures (Bernal et al. 2015, Chagaris et al. 2017, Sancho et al. 2018). Further research is warranted to determine aerobic scope in lionfish across a broader temperature range and up to their thermal maximum to establish a thermal performance curve that would improve predictions of how their invasion will be affected by ocean warming (Jensen et al. 2017).

3) *Metabolic phenotype*

In addition to temperature, we found an effect of metabolic phenotype on SDA. Lionfish appear to face a tradeoff between SMR and SDA, with higher SMR phenotypes having lower SDA with higher SDA_{peak} and shorter SDA_{dur} . This is somewhat consistent with the findings of Millidine et al. (2009), where juvenile Atlantic salmon (*S. salar*) with higher SMR had higher SDA_{peak} and shorter SDA_{dur} , however they also found that higher SMR phenotypes had a costlier SDA, whereas we found the opposite. This suggests that SDA_{peak} and SDA_{dur} may vary with SMR independently of SDA, but why SMR would act on these traits differently is unclear. Individual variations in SMR may be due to individual differences in relative organ size as found by Boldsen et al. (2013) in European eel (*Anguilla anguilla*), where individuals with larger intestines and livers had higher SMR, likely due to the energetic demands of these larger organs. This may explain why lionfish with high SMR phenotypes appear to digest meals more efficiently, rapidly, and with a higher SDA_{peak} , however such a relationship was found lacking by Norin and Malte (2012) in brown trout (*Salmo trutta*). Instead, they found that individual variation in SMR was explained by individual differences in enzyme activity in the liver, which if consistent in gastrointestinal organs could also explain the relationship between SMR and SDA we observed. Both processes may explain the differing effects of SMR on the cost of SDA versus its SDA_{dur} and SDA_{peak} observed between lionfish in this study and *S. salar* in Millidine et al. (2009), and further research is warranted to explore such questions.

We also observed a relationship between scope for activity and SDA, which to our knowledge has not been assessed in fish previously. Like with SMR, lionfish appear to face a tradeoff between scope for activity and SDA, with lower scope for activity phenotypes having a less costly SDA, shorter SDA_{dur} , and a smaller SDA_{peak} . Our finding that scope for activity did not vary with SMR but significantly increased with AMR suggests this relationship may have different mechanistic causes than that of SMR. It is possible that AMR is limited either by oxygen delivery by the cardiorespiratory system or by the maximum capacity of the mitochondrial electron transport system to utilize oxygen to produce ATP, traits which can vary among individuals (Metcalf et al. 2016, Norin and Clark 2016). One possibility is that inter-individual differences in relative organ sizes or enzyme activities may manifest in performance tradeoffs between organ systems (Boldsen et al. 2013, Norin and Malte 2012, Metcalfe et al. 2016), where lionfish with low scope for activity due to limited cardiac performance invest more in gastrointestinal organ mass and performance.

Regardless of its determinants, the finding that lionfish express intraspecific variation in metabolic phenotype and that it affects SDA could have important ecological implications. Intraspecific variation in SMR is thought to give fish populations persistence under varying food availability, as lower SMR individuals with lower energetic demands can weather periods of low food availability while higher SMR individuals can capitalize on periods of high food availability to grow and reproduce more quickly (Van Leeuwen et al. 2012, Metcalfe et al. 2016). Our finding that individuals with low-SMR phenotypes face up to a 33% greater energetic burden from SDA and an almost 8 hr longer digestive period may counter such an effect. Less is known about the ecological implications of intraspecific variation in MMR, or in our case, AMR (Metcalf et al. 2016). Having a two-fold phenotypic variation in scope for activity seems at odds with the sedentary nature of lionfish, however invasive lionfish were recently discovered to engage in long, energetically demanding agonistic interactions that likely determine dominance hierarchies (Fogg and Faletti 2018). Little is known about this behavior, however it suggests lionfish may sometimes compete for territory and access to prey. Lionfish with a low AMR, low scope for activity, and low-cost SDA would benefit from high prey densities that are typical of their invaded range and would not require them to compete

interspecifically (Chappell and Smith 2016, Hixon et al. 2016). When prey are more scarce, lionfish with a high AMR and high scope for activity may be better competitors and more able to secure access to prey that would offset their more costly SDA and longer SDA_{dur} , which we found to be up to 46% higher and almost 24 hr longer than in individuals with low scope for activity, respectively. As such, metabolic phenotypes may confer resilience to the lionfish invasion, particularly in light of past research showing they can cause local collapses in their prey populations and therefore variability in food availability (Green et al. 2012, Benkwitt 2015, Ingeman 2016, South et al. 2017).

4) Feeding frequency

Along with temperature and metabolic phenotype, we found an effect of feeding frequency on SDA that may manifest in ecologically relevant ways. Feeding frequently was less costly than feeding singularly, differing from the findings of Ross et al. (1992) and Guinea and Fernandez (1997) in Nile tilapia (*O. iuloticus*) and Gilt-head sea bream (*S. aurata*), respectively. These studies used meal sizes of 1% BM or less, which is below the 3.7% BM average and 8.5% BM maximum of this experiment, which may explain this difference. While little work has been done on this subject in fish, gut activity has been found to affect the cost of digestion in other ectotherms like lizards and snakes (Iglesias et al. 2003, Secor 2009), so the difference we observed may not manifest until meal sizes larger than those in Ross et al. (1992) and Guinea and Fernandez (1997) are used. In addition, we found that the cost of frequent feeding was more consistent than that of feeding singularly, possibly because of differing down-regulation of gut function during the 72 hr fast before SDA was recorded. The time required 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 hr (Montgomery and Pollak 1988). 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). This consistency has likely selected for frequent feeding in lionfish and concords with the high foraging rates observed in their invasive population (Côté and Maljković 2010, Green et al. 2011). Despite its lower and more consistent cost, frequent feeding posed a tradeoff to maintaining a scope for activity, as SDA_{peak} increased with shorter intervals from the previous meal. Norin and Clark (2017) found that such a tradeoff limited foraging

in a way that was not optimal for energy assimilation and growth in Barramundi (*Lates calcarifer*), suggesting frequent feeding may compound such an effect. While this is unlikely to affect lionfish because they readily exceed their scope for activity during feeding and digestion as discussed above, it illustrates the importance of energetic physiology in mediating feeding behavior and ecology in fish (Auer et al. 2015a, Auer et al. 2015b, Norin and Clark 2017).

Conclusion

In conclusion, we found physiological traits that would benefit lionfish as an invasive species, and as with past studies' findings may partially explain how they have become successful invaders (Wilcox et al. 2017, Côté and Smith 2018). Lionfish may be robust to and possibly benefit from some ocean warming by virtue of their digestive physiology, which concords with past physiological studies on lionfish (Cerino et al. 2013, Bernal et al. 2015, Dabruzzi et al. 2017) and other invasive species (Kelley 2014, Marras et al. 2015). Population and ecosystem models should use physiological characteristics and their relationship to environmental variables to predict how invasive species like lionfish impact native ecosystems, however some assumptions in such models are based on limited knowledge of species' physiology (Lennox et al. 2015). For example, an early bioenergetic model developed for lionfish (Cerino et al. 2013) that has subsequently been incorporated into ecosystem models of its invasion (Bernal et al. 2015, Chagaris et al. 2017, Sancho et al. 2018) used an assumed SDA_{coeff} that was almost double what we found in lionfish that fed frequently and reported a lower temperature-specific prey consumption rate than what we found in this study, which may have led these models to underestimate the prey consumption and ecological impact of invasive lionfish. In addition, we found that lionfish express inter-individual variability in metabolic phenotype and that this affects their SDA, which likely influences their behavior and ecology (Van Leeuwen et al. 2012, Auer et al. 2015, Metcalfe et al. 2016) and presents another potential shortcoming of invasive lionfish ecosystem models to date. The findings of this empirical experimental study and others like it may therefore improve such models, but further research is necessary to determine whether thermal plasticity affects the magnitude of our observed temperature effect, as well as to fully develop thermal performance curves for lionfish (Sandblom et al. 2014, Auer et al. 2016, Jensen et al. 2017).

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Figures

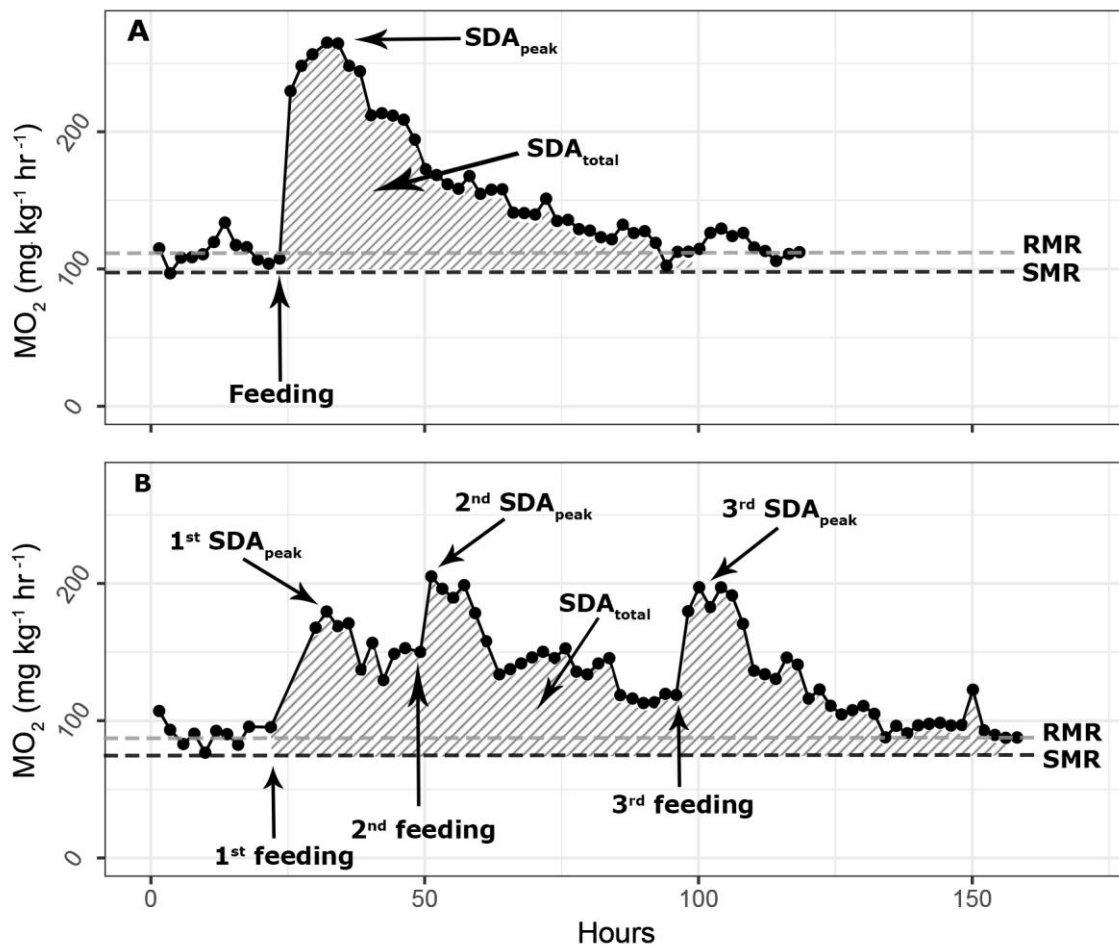


Figure 1. Examples of specific dynamic action (SDA) curves during single feeding (A) and repeated feeding (B). Metabolic rate data are Oxygen consumption rate values (MO₂, mg kg⁻¹ hr⁻¹) mass-adjusted to an 140 g fish ($b = 0.835$). SDA is the integral under the curve between postprandial MO₂ and standard metabolic rate (SMR, black line) over the duration between feeding and the third postprandial MO₂ value to fall below routine metabolic rate (RMR, grey line). Peak SDA (SDA_{peak}) is the highest unblocked postprandial MO₂ 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.

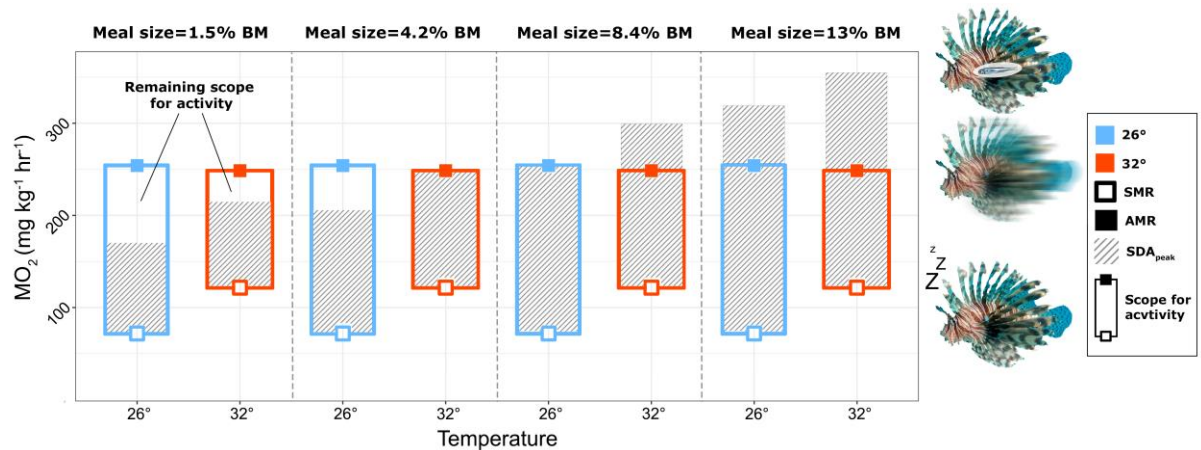


Figure 2. Relationships between peak specific dynamic action (SDA_{peak}), scope for activity, and temperature (26°C and 32°C ; $n = 13$ and $n = 16$, respectively) as a function of meal size for single meals. Metabolic rates are illustrated for lionfish at rest (SMR, bottom), at maximal activity (AMR, middle), and at maximal digestion of a large meal (SDA_{peak} , top). Metabolic rate data are mass-adjusted to an 140 g fish ($b = 0.835$). Meal size is measured as a percentage of body mass (%BM). Standard metabolic rate (SMR) and active metabolic rate (AMR) are Oxygen consumption rate values (MO_2 , $\text{mg kg}^{-1} \text{hr}^{-1}$). Plotted SMR and AMR values are averages from each temperature treatment. Scope for activity is the difference between AMR and SMR in an individual lionfish.

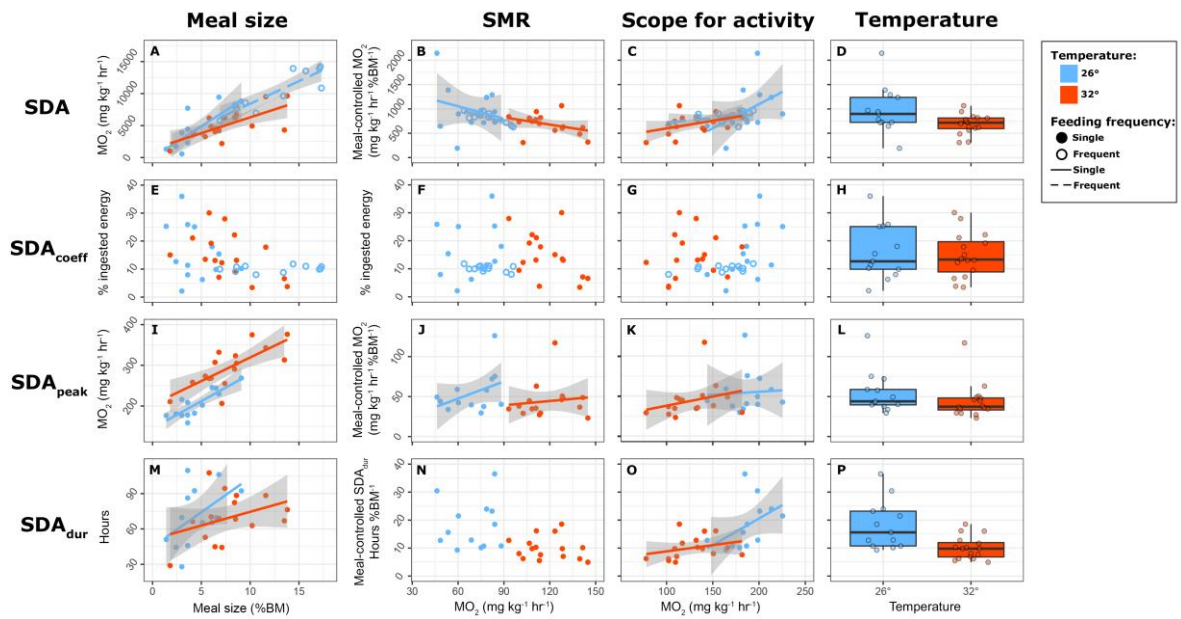


Figure 3. The relationship between specific dynamic action (SDA) and (A) meal size, (B) standard metabolic rate (SMR), (C) scope for activity, and (D) temperature; SDA coefficient (SDA_{coeff}) and (E) meal size, (F) SMR, (G) scope for activity, and (H) temperature; peak SDA (SDA_{peak}) and (I) meal size, (J) SMR, (K) scope for activity, and (L) temperature; SDA duration (SDA_{dur}) and (M) meal size, (N) SMR, (O) scope for activity, and (P) temperature in both single feeding (solid points and trendlines) and repeated feeding (empty points and dashed trendlines) experiments. Values derived from metabolic rate mass-adjusted to an 140 g fish ($b = 0.835$). $n = 13$ and $n = 16$ for the 26°C and 32°C , respectively, and $n = 13$ for the repeated feeding treatment. Trendlines with 95% CI included for significant relationships. Boxplots depict median (black bar), first and third quartiles (box hinges), and 1.5 interquartile range (whiskers). SDA, peak SDA (SDA_{peak}), and SDA_{coeff} are Oxygen consumption rate values (MO_2 , $\text{mg kg}^{-1} \text{hr}^{-1}$).

Data for SDA, SDA_{peak} , and SDA_{dur} in response to SMR (B, J, and N), scope for activity (C, K, and O), and temperature (D, L, and P) 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 active metabolic rate (AMR) and SMR in an individual lionfish.

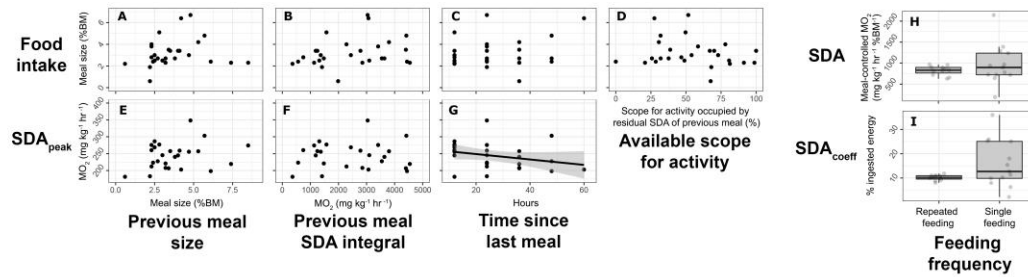


Figure 4. Relationships between specific dynamic action (SDA), food intake, and variables used in the repeated feeding experiment (A-D and F-H; $n = 13$), as well as the relationship between SDA, SDA coefficient (SDA_{coeff}), and feeding frequency (E and I). $n = 13$ for the repeated feeding treatment and $n = 13$ for the single feeding treatment. Trendlines with 95% CI included for significant relationships. Boxplots depict median (black bar), first and third quartiles (box hinges), and 1.5 interquartile range (whiskers). SDA, peak SDA (SDA_{peak}), and SDA_{coeff} are Oxygen consumption rate values (MO_2 , $mg\ kg^{-1}\ hr^{-1}$). Data for SDA 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). Previous meal residual SDA is the percentage of scope for activity occupied by SDA at the time of a repeated meal.

Supplementary Information

Table S1. Linear models and fits for SDA, peak SDA (SDA_{peak}), SDA coefficient (SDA_{coeff}), and SDA duration (SDA_{dur}). Equations represent relationships between non-transformed data.

Response variable	Model fit	Equation
SDA_{peak}	$r^2_{adj}=0.85$	$SDA_{peak}=12.5 \cdot \%BM+1.16 \cdot SMR+0.24 \cdot scope+25.8$
SDA	$r^2_{adj}=0.62$	$SDA=715.4 \cdot \%BM+17.2 \cdot scope-22.7 \cdot SMR-147.5$
SDA_{coeff}	$r^2_{adj}=0.14$	$SDA_{coeff}=-0.95 \cdot \%BM+0.03 \cdot scope+0.0036 \cdot SMR+13.04$
SDA_{dur}	$r^2_{adj}=0.31$	$SDA_{dur}=4.2 \cdot \%BM+0.23 \cdot scope-0.15 \cdot SMR+22.6$