ORIGINAL ARTICLE

Size affects digestive responses to increasing temperature in fishes: physiological implications of being small under climate change

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Abstract

Digestive metabolism is considered key to resilience of fish populations as it determines energy and nutrient availability for growth and survival. In cleaner fishes, digestion performance also influences the amount and the rate at which parasites can be removed from co-operating fishes, called hosts. Therefore, understanding the effect of temperature on digestive metabolic scope (*i.e.* the energy allocated to digestive processes) is crucial to predicting responses of fish communities to ocean warming. Body size can affect many physiologic processes and is thought to decrease with increasing temperature; therefore, we examined the effect of body mass and warming on digestive metabolic scopes in two sister species of cleaner gobies of the genus *Elacatinus* that reach different adult sizes. The dwarf-size *Elacatinus lobeli* increased digestive metabolic rates and scope while the larger *Elacatinus oceanops* decreased digestive metabolic scope with warming. Intra-specifically, larger *E. lobeli* also showed a decreased scope when compared to smaller individuals. Results from this study suggest that perhaps smaller fishes may have a digestive and metabolic advantage at higher temperatures and may be more resilient under warming temperatures.

Introduction

Cleaning symbiosis is an important relationship that plays a key role in maintaining the health of coral reef fish communities (Grutter 1999; Cheney & Côté 2005). In fact, cleaner fishes are known to reduce ectoparasite load, and sometimes scales and mucus, from the body surface, gills and mouth of larger co-operating fishes (commonly referred to as 'hosts', or more rarely 'clients') at specific sites on the reef, called cleaning stations (Grutter 1999; Grutter & Hendrikz 1999; Arnal & Côté 2000). This symbiotic relationship relies on cleaners' immunity against predation and consequently these fishes have evolved a stereotypical bright colored stripe along their body to allow hosts to easily recognize them (Colin 1975; Arnal & Côté 2000; Whiteman & Côté 2004a). Species that benefit from cleaning services range from herbivores to predatory fishes (Sazima *et al.* 2000). Because their role in reef community resilience is fundamental, the question of how or if cleaner fishes will be affected by climate change remains an important one to answer. It has been suggested that tropical organisms will be more sensitive to warming than their temperate counterparts because tropical species evolved in relatively thermally stable environments (Graham *et al.* 2007; Somero 2010; Donelson *et al.* 2011; Rummer *et al.* 2014). The metabolic scope (*i.e.* the energy allocated for activity beyond basic physiologic processes) of some tropical reef fishes decreases beyond an 'optimal temperature', thus limiting the efficiency of other important ecologic processes such as development, growth and reproductive capacity (Farrell *et al.* 2008; Rummer *et al.* 2014). In addition, tropical fishes are

thought to live close to their upper thermal limits, thus making them more vulnerable to even small (2–3 °C) increases in temperature. While locomotion is a commonly used index of aerobic performance in ectotherms (Batty & Blaxter 1992; Johansen & Jones 2011), an alternative way to assess the degree of biologic impairment caused by changes in environmental conditions in sluggish, site-attached fishes is to quantify the change in oxygen consumption rates during the specific dynamic action (SDA, or digestive metabolism; Hesthagen *et al.* 1995; Pang *et al.* 2011). In fishes, SDA occurs after feeding and represents the energy to ingest, digest, absorb and assimilate food (Von Herbing & White 2002; Pirozzi & Booth 2009; Tirsgaard *et al.* 2014). Metabolic rates exhibit a peak during SDA (MO_{2peak}), and termination of SDA is linked to the return of appetite in fish (Jobling 1981; Ferry-Graham & Gibb 2001; Papastamatiou & Lowe 2004; Sims *et al.* 2006; Di Santo & Bennett 2011a). As return of appetite is fundamental to trigger cleaning behavior (Arnal & Côté 2000; Sazima *et al.* 2000), and the amount of energy available for digestion and the duration of the SDA response are critical to feeding rates (Sims & Davies 1994; Pang *et al.* 2011), identifying the effect of temperature on digestive metabolism will improve predictions about future dynamics of cleaning symbiosis.

With the recent increase in temperature as a consequence of anthropogenic activities (IPCC, 2013), several studies have also started to document a decrease in body size across many species of ectotherms, including fishes (Daufresne & Boët 2007; Gardner *et al.* 2011). As body size directly correlates with metabolism, food requirements, and intra- and inter-specific competition for resources (Daufresne *et al.* 2009; Ohlberger *et al.* 2012), it is plausible that smaller fishes may gain an advantage under warming scenarios expected by the end of the century because they require less energy to survive. Smaller fishes tend to be more tolerant to warming-induced low oxygen levels in the environment and may, as a consequence, be more tolerant to increasing temperatures (Frederich & Pörtner 2000). Members of the genus *Elacatinus* are common cleaner fishes in the Caribbean (Sazima *et al.* 2000; Taylor & Hellberg 2006; Randall & Lobel 2009). Some live in monogamous pairs while others live in large groups and are highly social (Whiteman & Côté 2004a,b). Two sister species in this genus have similar shapes and general morphologies, but different adult sizes, the larger *Elacatinus oceanops* inhabiting coral reefs in the Florida Keys, and the dwarf-size *Elacatinus lobeli* living in the Belizean Meso-American Barrier Reef (Randall & Colin 2009). The two fishes were considered different morphs of the same species until genetic data confirmed size-related differences that supported a species-level designation (Taylor & Hellberg 2005, 2006;

Randall & Colin 2009). Here we tested the effect of body mass and temperature on digestive metabolic rates in adult cleaner gobies, and hypothesized that oxygen consumption during digestion would increase beyond routine rates in the smaller *E. lobeli* while it would decrease in the larger *E. oceanops* when ambient temperature is raised.

Methods

Study system and experimental design

Juvenile *Elacatinus lobeli* (n = 12) were collected at Wee Wee Caye, Belize (16°45′ N, 88°8′ W), while juvenile *Elacatinus oceanops* (n = 12) were collected in the Florida Keys, USA (25°9′ N, 80°17′ W) and held according to the approved Institutional Animal Care and Use protocol n. 11-041 at Boston University. Fishes were divided by species and randomly assigned to three acclimation groups containing four individuals each. All groups were maintained in well-aerated and filtered 130-l aquaria, containing artificial seawater (Instant Ocean and DI water). Water quality in each tank was monitored weekly to test for ammonia, nitrites and nitrates. Aquaria were kept at a diel photoperiod of 12 h light:12 h dark and temperature was initially set at 24 ± 0.5 °C with a submersible Ebo Jager 50-W aquarium heater. After a 2-week period at 24 °C, water temperatures were unchanged, or increased or decreased 0.5 °C per day until acclimation temperatures of 20, 24 and 28 °C were reached. These temperatures were chosen because they are experienced by both fishes in the wild (Table 1). Fishes were held at final acclimation conditions for a year before trials to ensure full acclimation to experimental conditions (Beitinger & Bennett 2000; Fangue & Bennett 2003) and allow the fish to reach adult size (*E. lobeli*: 1.30 ± 0.21 g, *E. oceanops*: 2.96 ± 0.18 g; Colin 1975; Randall & Colin 2009). Fish were fed a mixed diet of fresh frozen mysis shrimp and marine flakes twice daily *ad libitum* throughout the holding and acclimation period.

Measurement of routine and digestive metabolic rates

Oxygen consumption rates (MO_2 in $mgO_2 \cdot g^{-1} \cdot h^{-1}$) at pre-feeding state (routine metabolic rate, or MO_{2rout}) were determined at each temperature (*i.e.* 20, 24, 28 °C) using a custom-made closed respirometer (0.465 l) equipped with a circulating pump (Eheim, Deizisau, Germany; model 1043). Gobies (n = 4 per acclimation temperature, per species) were individually placed in the respirometer for 24 h to allow them to get accustomed to the experimental set up. During this time the

Table 1. Temperature (T) parameters (mean ± SEM) from two sites, Wee Wee Caye (WWC), Belize, and Key Largo, USA, between 2004 and 2014. Underlined mean pairs (between the two sites) are not statistically different (Student's *t*-test; α = 0.05).

month	mean T (°C)		mean T_{min} (°C)		mean T_{max} (°C)	
	WWC, Belize	Key Largo, USA	WWC, Belize	Key Largo, USA	WWC, Belize	Key Largo, USA
Jan	25.8 ± 0.3	22.0 ± 0.4	25.3 ± 0.3	21.6 ± 0.4	26.3 ± 0.3	22.5 ± 0.4
Feb	26.4 ± 0.3	22.5 ± 0.3	25.9 ± 0.3	21.8 ± 0.4	27.0 ± 0.3	22.7 ± 0.4
Mar	27.0 ± 0.2	23.5 ± 0.2	26.4 ± 0.2	23.1 ± 0.3	27.6 ± 0.2	24.0 ± 0.3
Apr	28.0 ± 0.2	25.8 ± 0.2	27.5 ± 0.1	25.3 ± 0.2	28.6 ± 0.1	26.1 ± 0.2
May	28.8 ± 0.1	28.0 ± 0.2	<u>28.3 ± 0.1</u>	<u>27.8 ± 0.2</u>	29.4 ± 0.2	28.6 ± 0.2
Jun	29.2 ± 0.2	30.0 ± 0.2	28.7 ± 0.2	29.7 ± 0.2	29.8 ± 0.2	30.5 ± 0.2
Jul	28.8 ± 0.1	30.6 ± 0.6	28.6 ± 0.2	30.3 ± 0.2	29.6 ± 0.2	31.0 ± 0.2
Aug	29.4 ± 0.2	31.0 ± 0.2	28.9 ± 0.2	30.7 ± 0.2	30.0 ± 0.1	31.4 ± 0.2
Sep	<u>29.6 ± 0.2</u>	<u>30.1 ± 0.2</u>	29.0 ± 0.2	29.9 ± 0.2	<u>30.2 ± 0.2</u>	<u>30.5 ± 0.2</u>
Oct	<u>28.7 ± 0.2</u>	<u>28.2 ± 0.2</u>	<u>28.2 ± 0.2</u>	<u>28.0 ± 0.2</u>	<u>29.3 ± 0.2</u>	<u>28.6 ± 0.3</u>
Nov	27.2 ± 0.2	24.8 ± 0.2	26.7 ± 0.2	24.5 ± 0.2	27.7 ± 0.2	25.2 ± 0.2
Dec	26.2 ± 0.3	23.3 ± 0.4	25.7 ± 0.3	23.0 ± 0.4	26.8 ± 0.3	23.7 ± 0.3
mean	27.8 ± 0.2	26.7 ± 0.2	27.3 ± 0.2	26.4 ± 0.2	28.4 ± 0.2	27.2 ± 0.2

respirometer chamber was kept open and water was continuously filtered and aerated to maintain normoxic conditions. The respirometer chamber was submersed in a temperature-controlled water bath, fitted with a Pro-ODO YSI oxygen probe, a valve to insert a pipette with food, and covered with dark plastic to reduce disturbance. Pre-feeding oxygen consumption (MO_{2rout}) was first measured for 30 min prior to feeding at 5-min intervals. After $MO_{2\ rout}$ measurements were completed, the respirometer chamber valve was opened, and fish were offered 0.5% of their body weight in food (mysis shrimp; National Research Council 1993) with a disposable pipette. In preliminary trials this amount of food represented the greatest quantity that both species of gobies would ingest without force-feeding. As force-feeding has been shown to influence digestion (Wetherbee *et al.* 1987; Di Santo & Bennett 2011a), all fishes in this study were allowed to feed voluntarily. If fish did not ingest food within a few seconds, the experiment was aborted. Food ingestion was confirmed by direct observation, and the respirometer valve was immediately closed and oxygen measurements resumed. Oxygen consumption during digestion was measured at 30-min intervals for 4 h. This time frame was chosen because preliminary trials revealed that oxygen consumption returned to routine levels after SDA within 4 h in these fishes. Dissolved oxygen in the respirometer never dropped below 80% saturation levels (Steffensen 1989). All metabolic rates were mass-adjusted using a scaling co-efficient of 0.9 as suggested by White *et al.* (2006) and White & Seymour (2011). Following each trial, blank respirometers were run for 1 h to adjust for background respiration, but oxygen decline was never detected in the empty respirometers.

Statistical analysis

We quantified the following parameters: MO_{2rout}, SDA_{peak}, digestive metabolic scope ($SDA_{peak} - MO_{2rout}$) and duration – calculated as the time period from feeding event to return of oxygen consumption rates to pre-feeding levels. The effects of species, size and temperature on metabolic parameters were determined using a full factorial three-way analysis of variance (ANOVA), after homogeneity of variance was confirmed. Return of metabolic rates to pre-feeding levels after MO_{2peak} was determined by repeated-measure ANOVA followed by Dunnett's test. All statistical decisions were based on α = 0.05. All statistical analyses were performed in JMP PRO version 11 (SAS Institute, Cary, NC, USA).

Results

Mean mass-adjusted MO_{2rout} were not statistically different between species at each temperature treatment (one-way ANOVA, P = 0.08) or among temperatures when analysed intra-specifically (one-way ANOVA, P > 0.05; Fig. 1). Following food ingestion, MO_2 increased to a peak after about 60 min in both species regardless of temperature before returning to pre-feeding levels (Fig. 1). SDA_{peak} was significantly affected by temperature, species and mass (three-way ANOVA, P < 0.0001; Fig. 2). In particular, increase in mass reduced SDA_{peak} across species (P = 0.0005) but we also observed interactions between species and temperature (P = 0.03). In *Elacatinus lobeli*, SDA_{peak} increased with temperature (P = 0.002) but decreased with mass (P = 0.01); we found no interactions between temperature and mass (P = 0.1). By contrast, in *Elacatinus oceanops* SDA_{peak} did
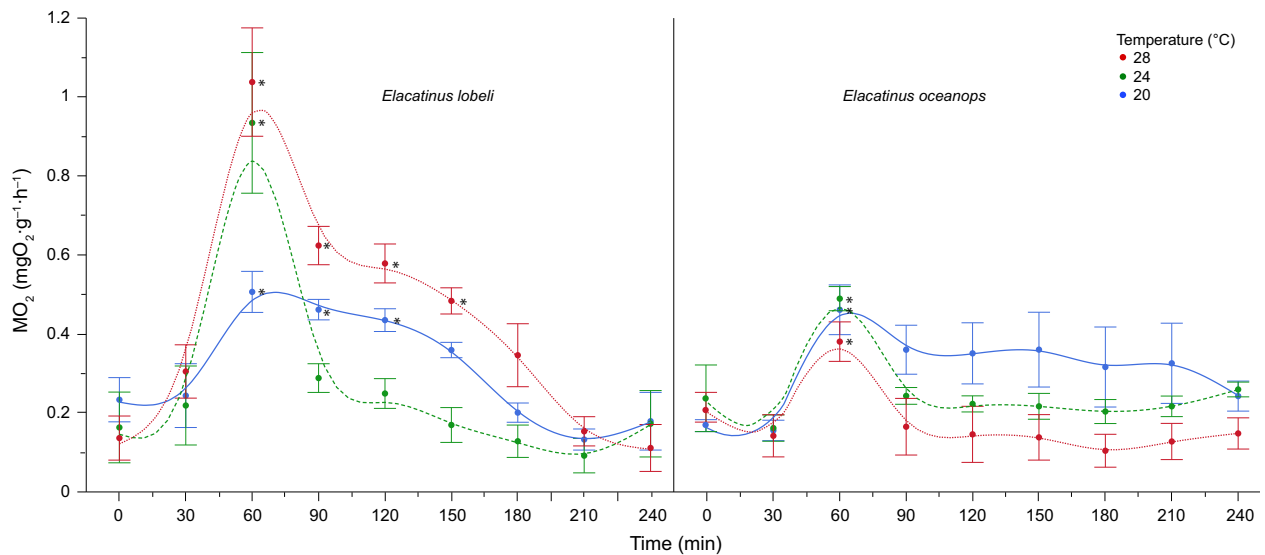
Fig. 1. Mass-adjusted oxygen consumption rates (MO_2 in $mgO_2 \cdot g^{-1} \cdot h^{-1}$: mean $\pm$ SEM) in adult *Elacatinus lobeli* and *Elacatinus oceanops* acclimated at three temperatures over time. Post-feeding (time > 0 min) MO_2 peaked for both species at 60 min, regardless of temperature (repeated-measures analysis of variance, P = 0.01, n = 12 per species). Asterisks indicate metabolic rates that differ significantly from pre-feeding levels (controls) at each temperature treatment (Dunnett's test, α = 0.05). Food ingestion occurred at time 0 after 30 min of routine oxygen consumption measurements.

not significantly change with temperature (P = 0.07) but decreased with body mass (P = 0.001). Within temperature treatments, larger body mass reduced the SDA_{peak} of *E. lobeli* at 28 °C (P = 0.02) and of *E. oceanops* at 20 (P = 0.006) and 24 °C (P = 0.02). Finally, digestive metabolic scope was significantly affected by temperature, species and mass (three-way ANOVA, P = 0.0001), with the strongest factor being species (P = 0.005; Fig. 3). In *E. lobeli*, higher temperatures significantly increased digestive metabolic scope (P = 0.02) but mass had no significant effect (P = 0.2). In *E. oceanops*, only temperature had a significant effect on digestive metabolic scope by reducing energy available for digestion (P = 0.004). The total net amounts of oxygen used during SDA (after subtracting the routine oxygen consumption) were: 1.09 ± 0.13 (at 28 °C), 0.45 ± 0.11 (at 24 °C), 0.35 ± 0.12 (at 20 °C) $mgO_2 \cdot g^{-1}$ in *E. lobeli* and 0.05 ± 0.03 (at 28 °C), 0.15 ± 0.02 (at 24 °C), 0.15 ± 0.04 (at 20 °C) $mgO_2 \cdot g^{-1}$ in *E. oceanops*. Time to return to MO_{2rout} levels after MO_{2peak} differed between the two species (Fig. 1).

Discussion

This study aimed to evaluate the effect of temperature and size on routine and digestion metabolism in two sister species of cleaner gobies of the genus *Elacatinus*. We found that temperature had a significant effect on digestive metabolic rate and scope but that this differed

between the species. The larger *Elacatinus oceanops* decreased digestive metabolic scope while the smaller *Elacatinus lobeli* increased it at the highest temperature (28 °C). Generally, body mass had a significant effect on digestive metabolic rates by increasing metabolic scope at 20 and 24 °C, and decreasing it at 28 °C. Body temperature in aquatic ideal poikilotherms, such as fishes, closely matches their thermal environment; and most physiologic processes are profoundly influenced by changes in temperature (Fry & Hart 1948; Fry 1971; Magnuson *et al.* 1979; Di Santo & Bennett 2011b; Pang *et al.* 2011). In particular, digestive processes are known to be sensitive to temperature in fishes that experience thermally fluctuating environments (Wurtsbaugh & Neverman 1988; Neverman & Wurtsbaugh 1994; Sims *et al.* 2006; Wells *et al.* 2009; Di Santo & Bennett 2011a). Nonetheless, it is not unusual to observe low sensitivity of metabolic rates to a range of temperatures in some fishes especially if they have evolved in relatively stable thermal environments (Windell *et al.* 1978; Di Santo & Bennett 2011a,b).

Although the sample size per treatment was low (n = 4 per species), the results from this study still show differences in digestive metabolic responses in sister species that differ in adult size and that were acclimated at the same temperatures. In fact, warming reduced the digestive scope of *Elacatinus oceanops* but enhanced it in *Elacatinus lobeli*. For both species, post-prandial metabo-

Fig. 2. Specific dynamic action peak (SDA$_{peak}$) decrease (mgO$_2$·g$^{-0.9}$·h^{-1}) with mass (g) in *Elacatinus lobeli* and *Elacatinus oceanops* at each temperature treatment (three-way analysis of variance, P < 0.0001, n = 12 per species). Within species, a significant correlation between body mass and SDA$_{peak}$ was found at 28 °C in *E. lobeli* (P = 0.02) and at 20 and 24 °C in *E. oceanops* (P = 0.006 and 0.02, respectively).
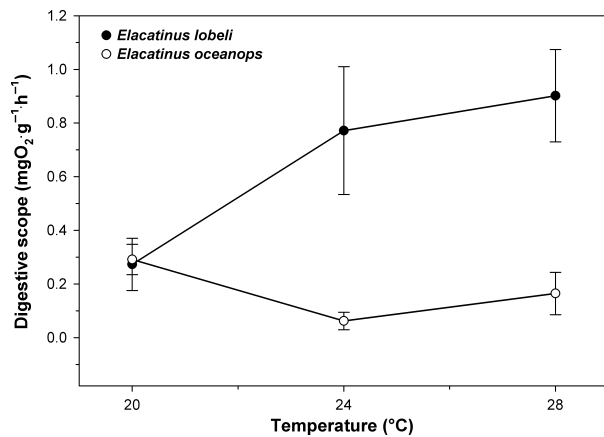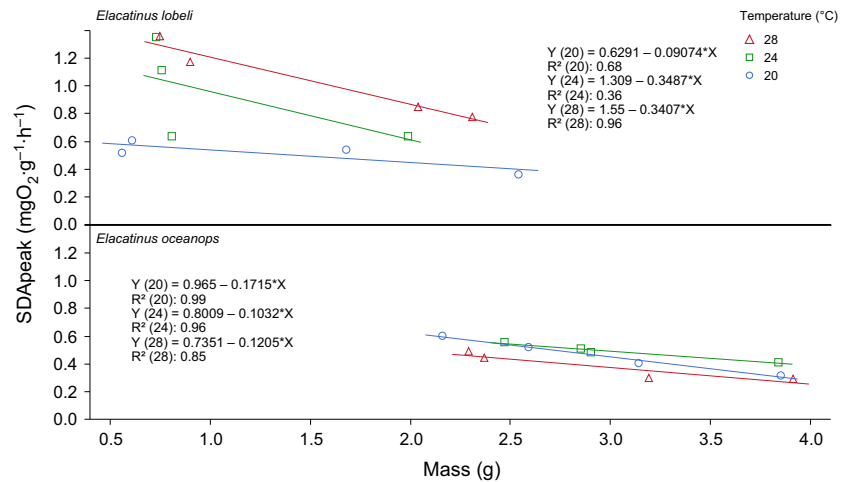

Fig. 3. Digestive scope (peak specific dynamic action – routine metabolic rates; in mgO$_2$·g^{-1}·h^{-1}: mean ± SEM) in adult *Elacatinus lobeli* and *Elacatinus oceanops* acclimated at three temperatures. Temperature significantly affects the digestive scope in both species (three-way analysis of variance, P < 0.01, n = 12 per species).

lism peaked after about 60 min of ingestion regardless of temperature, suggesting a temperature-insensitive but time-dependent increase in digestive activity. Digestive peak metabolism (MO$_{2peak}$) was significantly affected by body mass in both species. Additionally, *E. oceanops* only showed a clear peak in digestive metabolism at 24 °C, and exhibited high variability in MO$_{2peak}$ over time at 20 and 28 °C. These results are not unusual, as coral reef fishes living close to the limit of their thermal optimum often show high variability in responses to threshold temperatures (Rummer *et al.* 2014). According to the oxygen capacity limited thermal tolerance hypothesis advanced by Pörtner & Farrell (2008), reduced thermal tolerance windows are associated with a mismatch between oxygen demand and supply at high temperatures. In this case, the post-prandial metabolic rates of fishes acclimated at

28 °C would be determined by the capacity of the cardio-respiratory system to deliver oxygen to tissues (Pörtner & Farrell 2008). At high temperatures, oxygen becomes a limiting factor and sets the upper limit of digestive metabolisms in aquatic ectotherms (Pörtner *et al.* 2006; Kassahn *et al.* 2009). Beyond their thermal optima, at *pejus* temperatures (where metabolic performance transitions from its optimum to an increasingly impairing condition; Frederich & Pörtner 2000), fish increase energy demand to re-establish ion balance, thereby limiting the capacity to further enhance digestive performance (Jobling 1981; Pang *et al.* 2011). Variations in digestive performance and decline above thermal optima were also analysed in different populations of killifish (McKenzie *et al.* 2013), suggesting that warming may exert a markedly negative effect on key aerobic performances such as digestion in tropical fishes. In the present study, *E. oceanops* only showed a clear peak in digestive metabolism at the mid temperature of 24 °C, suggesting that this may approximate the thermal optimum for digestion in this species. Conversely, *E. lobeli* exhibited an increase in metabolic scope at the highest temperature as well (28 °C), implying that metabolic capacity can be enhanced at high temperatures in this smaller goby. Given these results we predict that smaller fishes have the potential to obtain a significant digestive advantage by occupying warming environments, but more species-systems need to be tested.

The duration of the SDA response has been shown to decrease with increasing body mass in juvenile Atlantic cod *Gadus morhua* (Von Herbing & White 2002). Although prolonged contact of food with digestive surfaces is known to enhance nutrient absorption (Di Santo & Bennett 2011a), this strategy is thought to be more beneficial to intermittent feeders such as large predators (Sims *et al.* 2006; Di Santo & Bennett 2011a). For contin-

uous feeders like cleaner gobies, faster digestion rates and therefore quicker return of appetite may be more advantageous than more efficient but discontinuous food absorption (Wurtsbaugh & Neverman 1988) as these fishes remove parasites from their hosts (and therefore, digest) throughout the day. Cessation of the SDA response is key to return of appetite and initiation of feeding in fishes (Sims & Davies 1994). It is therefore possible that further warming might increase *Elacatinus oceanops* cleaning rates, because digestive processes will be less efficient at higher temperatures. However digestion efficiency was not measured in this study and further work should include this measurement. Although potentially negative for the fish *per se*, because it increases the need to seek food more frequently, higher cleaning rates could favor parasite removal, and therefore enhance the health of coral reef fishes. Further field studies could reveal if there is indeed a correlation between daily as well as seasonal temperatures and cleaning rates in these two species.

When just body mass was considered across species, digestive scope significantly increased at the lowest temperatures tested in larger fishes while it decreased at the highest temperature. However, when species were analysed separately only *Elacatinus lobeli* showed a decrease in digestive scope with increasing size. It is possible that digestive metabolic scopes decrease beyond a 'threshold size' in these cleaner fishes so only the largest individuals of the dwarf-size *E. lobeli* are penalized by reaching a larger size. This pattern is consistent with the temperature-size rule observed in wild populations that describes a general pattern of decrease in body size with increasing temperature (Angilletta 2009; Daufresne *et al.* 2009). Although it is well known that body size affects physiological processes, most studies (with a few exceptions, for example Di Santo 2015) have analysed single species, compared different life stages, or locations so could not control for localized effects on body size (Daufresne *et al.* 2009; Gardner *et al.* 2011).

Reduction in fish body mass as a consequence of warming can be the product of different processes. This outcome could be achieved through a shift in size-at-age (observable in adult individuals) and/or through an increase in juveniles in the population. It is possible that in reality both processes are occurring. Older and larger eelpout showed a reduced tolerance to warming when compared to younger and smaller individuals (Pörtner 2001, 2002; Pörtner & Farrell 2008). It is also plausible that smaller adult ectotherms may be better able to cope with warming because of the reduced mismatch between oxygen-demanding tissues and delivery by the cardio-respiratory system (Pörtner 2001). Therefore, the results from this study point out that in warming environments smaller fish may gain a physiological advantage during digestion when compared to larger individuals. As a consequence, as oceans warm, we might observe a shift towards smaller cleaner fishes on reefs. The population dynamics of keystone species, such as cleaners, are crucial in determining ecosystem resilience and stability (Sazima *et al.* 2010; Rossi *et al.* 2013). As fish usually experience satiation after ingesting a given percentage of their body weight in food, it is most likely that smaller individuals may only be able to remove a fraction of the parasite load that a larger conspecific or congeneric is capable to ingest. At this point, it is still unknown if cleaner gobies will be able to increase the number of individuals in each colony to compensate for a reduced biomass of ectoparasites removed from fishes by each individual; however, understanding if any compensatory mechanism will take place is crucial to predicting the future of healthy reefs.

Conclusions

Our study on two sister species of cleaner gobies suggests that smaller individuals might gain a physiological advantage during digestion as temperature increases. These results corroborate previous findings that linked a broad shift of body size in ectotherms to global warming. As larger individuals are likely to remove more parasites from hosts than smaller conspecifics or congenerics, it is likely that coral reef fishes might experience an increase in parasite load, unless a small body size is ecologically compensated by a higher density of individuals in cleaner fish colonies.

Acknowledgements

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