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REPORT

Thermal sensitivity of juvenile rabbitfishes *Siganus doliatus* and *S. lineatus* (Siganidae): a key role for habitat?

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Abstract The majority of our understanding of the effects of climate change on coral reef fishes are currently based on studies of small-bodied species such as damselfishes. By contrast, we know little about the potential impacts of ocean warming on larger species of herbivorous and detritivorous reef fish, despite them being a critical functional group and an essential source of food protein for millions of people. In addition, we know little of the role of habitat in determining species' thermal sensitivity and the legitimacy of extrapolating thermal performance across closely-related species from different habitat types. Here we test the effect of exposure to increased water temperature during juvenile development on key physiological and behavioral traits of two species of rabbitfish typically associated with different habitats: *Siganus doliatus* (reef-associated) and *S. lineatus* (estuarine). Wild-caught juveniles were reared for 14 weeks at temperatures representing present-day ambient conditions (28.0 °C), late-summer ambient conditions (30.0 °C), or those projected on reefs under future global warming scenarios (31.5 °C). We then measured the somatic (growth, condition, immune response) and behavioral (feeding rate, latency to feed and activity level) traits of individuals within each treatment to determine the sensitivity of each species to elevated water temperatures. Overall, both species showed comparatively robust levels of thermal tolerance, based on previously-documented responses of small-bodied reef fishes. However, two very different patterns emerged. The reef-associated *S. doliatus* showed a greater physiological response to temperature, with negative effects on hepatosomatic condition and immune function observed in individuals exposed to the 31.5 °C treatment. By contrast, there were no negative physiological effects of temperature observed in *S. lineatus* and instead we recorded behavioral changes, with individuals at 30 °C and 31.5 °C displaying altered feeding behavior (increased feeding rate and decreased latency to feed). These distinct responses observed between congeners are likely due to their evolutionary history and flag the potential inaccuracies that could arise from extrapolating effects of ocean warming across even closely-related species adapted to different habitats.

Keywords Climate change · Ocean warming · Physiological condition · Immunity · Feeding · Behavior

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Introduction

Climate change is one of the most prominent threats to biodiversity and ecosystem processes currently facing our planet (Parmesan et al. 2000; Munday et al. 2008a; Hobday and Lough 2011; Boyles et al. 2011; Crozier and Hutchings 2014; GBRMPA 2019) with coral reefs in the vanguard of affected habitats. In recent years, coral reefs globally have been exposed to periods of elevated ocean temperatures above the normal summer ranges (i.e., marine heatwaves), which have severely impacted reef building corals via bleaching, mortality and increased disease prevalence

(Hoegh-Guldberg et al. 2007; Hughes et al. 2017, 2018; King et al. 2017), negatively impacting other reef-associated animals (Bernal et al. 2020). Marine heatwaves are predicted to increase in frequency (King et al. 2017; IPCC 2019) along with projected increases in tropical average sea surface temperature of 1–2 °C by 2100 (Hobday and Lough 2011; GBRMPA 2019; IPCC 2019). Since tropical reef organisms have evolved in a relatively thermally stable environment (Hoegh-Guldberg et al. 2007; Tewksbury et al. 2008), it is expected that they will be impacted by temperature increases and associated thermal stress.

Ocean warming is likely to disproportionally affect marine ectotherms as they lack internal temperature regulation and, as a result, exhibit thermal performance that is strongly linked to the range of temperatures normally experienced (Pörtner 2001; Sunday et al. 2011; Rodgers et al. 2018). Increases in water temperature of only 1–3 °C during summer can negatively affect tropical reef fish, due to their narrow thermal performance range, and the fact that they are already living close to their upper thermal limits (Gardiner et al. 2010; Rummer et al. 2014; Rodgers et al. 2018; Pinsky et al. 2019). Thermal effects occur from the cellular to the whole organism level and, in the case of the latter, effects can be expressed as physiological or behavioral changes (Munday et al. 2008a, 2009; Hoey et al. 2016). In terms of physiology, elevated temperature increases the rate of cellular processes and consequently metabolic rates (Gardiner et al. 2010; Nilsson et al. 2010; Johansen and Jones 2011; Norin et al. 2014), with extreme temperatures disrupting enzymatic function (Sabapathy and Teo 1994; Boyles et al. 2011; Madeira et al. 2013). These physiological effects then can impact energy budgets, with associated negative effects on growth, development rate, reproduction and survival (Munday et al. 2008b; Donelson et al. 2010; Zarco-Perelló et al. 2012; Hoey et al. 2016; Rodgers et al. 2018). In addition, the immune system of fishes is directly exposed to changes in external temperature. This means that immune function is likely to be highly susceptible to changes in water temperature, with potential consequences for the spread of infectious diseases among fish populations under thermal stress (Dittmar et al. 2014). For example, previous research has demonstrated that simulated heatwave events cause immune disorders in stickleback (Dittmar et al. 2014) and that the combination of high water temperature and a compromised immune system has a negative effect on survival of wild gudgeon (Petitjean et al. 2020). Animal behavior, of course, also has the potential to be sensitive to environmental stimuli. In fact, the assumed lower costs of behavioral plasticity, mean that behavior is typically considered the first line of response for an individual experiencing environmental change (Clark et al. 2013; Wong and Candolin 2015; Habary et al. 2017). For example, individuals experiencing higher metabolic costs associated with increased daytime temperature can increase foraging rate (Nowicki et al. 2012).

Whether the effects of temperature are expressed through physiology or behavior, organisms experience the greatest sensitivity to thermal stress during their larval and juvenile stages (Houde 1989; Green and Fisher 2004; McLeod et al. 2013; Munday et al. 2013). Temperatures experienced during early life can have lasting effects, impacting on later life fitness either in terms of survival or reproductive success (Angilletta et al. 2003; Donelson et al. 2011; Munday 2014; Hoey et al. 2016). With few exceptions (Pratchett et al. 2013; Johansen et al. 2015; Clark et al. 2017), and those studies conducted for aquaculture purposes (Castillo-Vargasmachuca et al. 2017; Larios-Soriano et al. 2020; Mazumder et al. 2020), research to date in this field has focused on small, site-attached species such as damselfish (Pomacentridae) as they are easy to keep and rear in captivity (Pratchett et al. 2011; Munday 2014). Not only this, but our understanding of the role that habitat-based adaptation of thermal limits might play in driving variation in thermal sensitivity within- and between-species is currently limited by a lack of studies adopting an inter-habitat comparative approach. The thermal sensitivity of a species or population is likely to reflect the thermal range of that habitat: species living in habitats with broader thermal ranges may be able to cope with greater increases in mean temperature (Bennett et al. 2019). Experimental tests of this hypothesis, however, remain limited, impacting on our ability to accurately predict the likely impacts on coral reef fishes of future climate scenarios.

Herbivorous fishes are critical to the maintenance of reef health via their roles in removing algae (grazing of turf algae and browsing of macroalgae) and enhancement of coral larvae settlement (scraping of reef pavement that opens up settlement space) (Hughes et al. 2003, 2007; Bellwood et al. 2004; Cheal et al. 2010; Rasher et al. 2013). Exclusion of herbivores, both as part of controlled experiments and via overfishing, has been shown to reduce reef resilience, potentially resulting in habitat phase shifts to alternative stable states such as macroalgal dominance (Hughes et al. 2007; Cheal et al. 2010; Graham et al. 2015). Despite their critical role in reef ecosystem functioning, and the fact that many herbivore species are targets of commercial and subsistence food fisheries around the globe, little work has been done to investigate their sensitivity to environmental change and their potential to acclimate or adapt to rising ocean temperatures. Within the herbivore guild native to Indo-Pacific reefs, rabbitfish (Siganidae) is an important group that is essential to reef health and resilience via their role in removal of algae (Fox and Bellwood 2008; Cheal et al. 2010), as well as their microhabitat feeding preferences (Fox and Bellwood

2013). To date, only two studies have examined the thermal sensitivity of rabbitfish, *Siganus rivulatus* and *Siganus canaliculatus*, finding that their growth and function of a key digestive enzyme (amylase) were affected by elevated temperatures (Sabapathy and Teo 1994; Saoud et al. 2008).

In this study, we investigated the thermal sensitivity of two closely-related species of rabbitfish from different habitats, *Siganus doliatus* (reef-associated) and *Siganus lineatus* (estuarine and mangrove-associated), over a 3 month period of their juvenile development. New recruits of both species were exposed for 14 weeks to one of three temperatures, representing present-day summer ambient conditions (28.0 °C), late-summer ambient conditions (30.0 °C), or those of projected ambient summer conditions under future global warming (31.5 °C, Hobday and Lough 2011). At the end of this period, we recorded traits related to the overall somatic condition of individuals (growth, hepatosomatic index and immune function). We also measured key behaviors exhibited by individuals within their respective temperature treatments (feeding rate and activity level) to determine each species' potential to exhibit behavioral plasticity when under thermal stress. We hypothesized that juvenile *S. lineatus* would show lower sensitivity to thermal conditions during development, given their primary association with estuarine and mangrove habitats that typically experience higher fluctuations in water temperature over the diel cycle than the reef-edge habitats of juvenile *S. doliatus* (Wolanski et al. 1981; Whitfield 1999; Harrison and Whitfield 2006; Childs et al. 2008). For example, while summer temperatures of inshore reef areas of the mid-Great Barrier Reef region and estuarine waters within the same range show averages of 28–30 °C, these estuarine waters can exhibit temperature peaks of 33–34 °C in some restricted areas (Kenny 1974; AIMS 2017; Waltham and Sheaves 2017; DAFF 2018). We also hypothesized that *S. lineatus* would exhibit greater potential for behavioral plasticity than *S. doliatus*, given its known ability to display plasticity in feeding activity patterns over the diel cycle, with mangrove populations feeding diurnally and reef populations feeding nocturnally (Fox and Bellwood 2011).

Material and methods

Ethical note

This research was conducted under approval A2610 authorized by James Cook University's Animal Ethics Committee. Fish were collected from the wild under permits from the Great Barrier Reef Marine Park G18/40957.1 (Fox) and James Cook University's limited impact research permit for the Great Barrier Reef Marine Park (CSE approval 103, Donelson and LaMonica), as well as Queensland Fisheries DAF 200,552 (Hoey).

Study species

The barred spinefoot rabbitfish, *Siganus doliatus* (Guérin-Méneville 1829–38), is a tropical reef fish found throughout the Western Pacific (Woodland 1997). Adults inhabit coral-rich areas feeding predominantly on turfing algae and reach a total length of 20–25 cm (Woodland 1997; Kuiter and Tonozuka 2001; Fox et al. 2009; Hoey et al. 2013). Juveniles form loose schools across reef flats and within habitat-structuring macroalgae such as *Sargassum* spp. (Woodland 1997; Kuiter and Tonozuka 2001; Tang et al. 2020). The golden-lined spinefoot rabbitfish, *Siganus lineatus* (Valenciennes 1835), is a tropical, reef-associated species that feeds predominantly on detritus (Fox et al. 2009), but also ingests algae, sediment and cyanobacteria (Woodland 1997; Fox et al. 2009; Hoey et al. 2013). *S. lineatus* can reach total lengths of 25–43 cm, inhabiting shallow coral lagoon areas and mangroves as adults (Kuronuma 1961; Lieske and Myers 2002; Woodland 1997). Juvenile *S. lineatus* are most typically found within estuarine and mangrove habitats (Woodland 1997), although have also been documented in nearshore macroalgal beds (Tang et al. 2020).

Fish collection and husbandry

On March 8th 2019, we collected a total of 50 juvenile *S. doliatus* from *Sargassum* spp. patches at 1.5 m water depth within Hazard Bay, Orpheus Island (18.6331°S, 146.4900°E) using a barrier net (25 m × 2 m, Line 8, 2.5″ mesh size) and clove oil. During the period March 20th–April 2nd 2019, 48 juvenile *S. lineatus* were collected from estuarine habitats along the Ross River, Townsville (19.5280°S, 146.8344°E) using a cast net (2 m drop diameter and 2 cm mesh size). We were unable to use seine netting methods targeting smaller *S. lineatus* individuals due to University safety regulations (crocodile risk). For both species, live fish were transported on the day of capture to the Marine and Aquaculture Research Facilities Unit (MARFU) at James Cook University, Townsville, with constant aeration. Fish underwent a formalin seawater treatment on arrival at MARFU (immersion in seawater containing a solution of 250 ppm formalin (37% w/w formaldehyde) for no less than 45 min, JCU MARFU SOP AQU-031) to dislodge any parasites, before being allocated to 32 L holding tanks.

Fish were habituated to experimental aquaria in small groups of 3–5 individuals per 32 L tank maintained at the ambient control water temperature of 28.0 °C for 7–18 days with a continuous flow of seawater from a

closed recirculating system (~ 7500 L) fitted with filtration, UV sterilization and protein skimmer. Seawater in the experiment underwent particle filtration (2 × 50 micron bag filters) before being UV sterilized, chilled or heated (as required) and supplied to tanks. The light regime was on a 12 h light: 12 h dark schedule similar to the summer average of the collection region. Correct functioning of the aquarium systems (pumps, water flow, temperature, pH) was constantly monitored and controlled through direct digital control systems (OMNI Innotech). The water quality parameters salinity, pH and nitrates were also measured weekly by MARFU technical staff. Once fish were observed to be feeding well, they were measured (see below for details) and allocated to individual 32 L tanks containing two PVC pipes (8–13 cm length, 5–9 cm diameter) for shelter. All tanks were covered with plastic mesh to prevent fish escaping. Throughout the experiment fish were fed ad libitum twice daily. Food sources were live green algae (*Caulerpa* and *Ulva* spp.), marine green (protein 61.5%, algae/vegetables 30.8%, fiber 7.7%) and fish pellets (INVE O.range NRD 5/8; protein 55%, lipid 9%, fiber 1.9%, moisture 8%). Tanks were cleaned weekly by siphoning excess food and waste.

Temperature treatments

Fish in individual tanks were randomly allocated into one, of three temperature treatments: 28.0 °C, 30.0 °C or 31.5 °C (n = 16 per species per temperature, with n = 15 for *S. doliatus* at 31.5 °C). Experimental temperatures were selected based on ambient summer temperatures experienced on reefs of Orpheus Island (central region of the Great Barrier Reef). Early summer temperatures for this region (October-December) are an average of 28.0 °C, while later summer months (February–March) can regularly reach an average of 30.0 °C (AIMS 2017). The warmest treatment of 31.5 °C was experienced in the Orpheus Island area during the heatwaves of 2016–2017 (Hughes et al. 2017, 2018; Veilleux et al. 2018; Eakin et al. 2019). These warmer temperatures of 30.0 °C and 31.5 °C also coincide with expected average increases in ocean temperature of the region due to climate change by the year 2070 (Hobday and Lough 2011). Temperatures were maintained ± 0.05 °C of the desired set points (controlled via MARFU OMNI system and confirmed via spot checks with a Comark C22 temperature probe twice daily). Individuals were held at their respective temperature treatment for 14 weeks.

Growth and development measures

At the start and end of the experiment, juvenile fish were placed in a transparent plastic bag filled with a small amount of seawater and photographed (Canon G15). A 5-mm-grid scale was placed in the background of each photo. Photographs were later analyzed using ImageJ (version 1.52a) to determine standard length and body depth (measured from base of the first dorsal fin spine to the anterior edge of the pelvic fin) to the nearest 0.01 mm (average of 3 measurements). Fish were also weighed before and after temperature treatments (Sartorius BL 150 S scale) to the nearest 0.001 g. Growth was calculated as the change in standard length, body depth and mass over the experimental period.

At the conclusion of the experiment, fish were euthanized via pithing with their livers dissected out and weighed to the nearest 0.0001 g. Hepatosomatic index (HSI) was calculated as a proxy of energy reserve and physical condition by dividing liver mass (g) by final total body mass (g) (including liver) of each individual fish and multiplying by 100 to get a percentage. Sagittal otoliths were then extracted from a subset of individuals from each species (*S. doliatus* n = 13, *S. lineatus* n = 13) for age analysis. Otoliths were embedded in resin and mounted on glass slides, ground and polished with increasingly fine sand and polishing paper (800, 1200 and 4000 grit) and viewed under a dissection microscope (Olympus BX41). Daily growth increments (rings) were counted on two separate occasions with at least 7 days between counts, and the highest count was used (JMD). All observations were made blind to species, temperature treatment and previously recorded counts for that particular otolith. Rings were counted to the core and a settlement ring (where visible). If settlement was not clearly visible, the published average 1 month pelagic larval duration (PLD, *c.f.* Taylor et al. 2017) was removed from the count. Visible settlement rings corroborated this ~ 1 month PLD.

Immune response

In order to examine the sensitivity of the immune system of *S. doliatus* and *S. lineatus* to thermal stress, we carried out an immune challenge on all individuals. After 11 weeks of exposure to their respective treatments, we measured the immune response of each individual (*S. doliatus* n = 36; *S. lineatus* n = 45) using a phytohaemagglutinin (PHA) injection assay to elicit body inflammation as a proxy for a cell-mediated immune response (O'Connor et al. 2014). Since this was the first time that this method has been used to quantify immunity in the two study species, we also conducted separate control injections of phosphate buffer saline solution (PBS) in order to validate the PHA assay methodology for *S. doliatus* and *S. lineatus*. For both sets of trials, fish were first anesthetized in a seawater solution of clove oil (0.1 ml clove oil: 1 l seawater). While under anesthesia, three consecutive measurements of caudal

peduncle thickness were taken using pressure controlled calipers (Mitutoyo Absolute AOS Digimatic Caliper; accuracy: 0.1 mm), with the average of the three measurements to be used in subsequent analyses (see *Statistical Analyses*). We then injected a fixed volume (0.2 ml) of either PHA (dissolved in PBS at 0.01 mg: 0.01 ml) or the control PBS. Fish were immediately returned to their individual aquaria and then re-measured 24 h after injection (again recording the average of three consecutive measurements). Immune response was calculated as the difference between pre- and post-injection measures. In order to obtain adequate sample sizes for the control trials, all fish underwent the experimental PHA trial (with associated re-measurement of the injection site 24 h later) followed, at least 4 d later, by the PBS control injection (with associated re-measurement of injection site after 24 h). Note that tissue swelling at the injection site (the lateral thickness of the caudal peduncle) disappears after 72 h (Iglesias-Carrasco et al. 2019a, b), meaning that measurements taken in the second, PBS control test would not be impacted by any immune response previously recorded. A single investigator performed all measurements for consistency (LEL), with data transcribed by a second individual to ensure that the measurer was blind to pre-injection values. In addition, all data were recorded blind with respect to temperature treatment. To confirm the absence of a response to injection with PBS, we ran one-sample t-tests for each species, with the null hypothesis that mean swelling response = 0.

Behavioral assays

Behavioral assays were conducted on each individual after 10 weeks of exposure to their respective temperature treatment. The day prior, tanks were cleaned, two PVC shelter pipes were placed in the back, left corner, and a 20-cm-long 4-mm-diameter tube was adhered to the opposite upper corner of the tank. Fish were starved for 15 h prior to the commencement of trials, which were conducted between 0900 and 1000 h and 1030–1130 h, in keeping with their usual feeding regime (equal numbers of fish were randomly assigned to each trial time). At the start of each trial, a GoPro Hero 3 was placed below the 20 cm tubing in the corner of the tank and set to record for a period of 60 min (the first 20 min of each recording was treated as acclimation to the presence of the camera and was excluded from all subsequent analyses).

Following the 20 min period of acclimation, we commenced observations on the activity level of each fish. Each trial lasted for 20 min, during which time we recorded the amount of time (in s) fish spent either hiding in the shelter of the PVC piping, or out swimming in the tank. The amount of time fish were out of view of the recording was also noted. Two individuals (both of them *S. doliatus*) that were not visible for over 1000 s of the video were excluded from further analysis. The proportion of time spent inside versus outside the shelter was calculated as a proxy for activity level. At the conclusion of the activity level trial (40 min into recording), a solution of 0.5–0.6 g of marine green with 5 ml of seawater was discretely injected through the 20 cm tube above the GoPro. Although *S. doliatus* and *S. lineatus* are known to have distinct diets in the wild, they did not show differential preferences for the artificial foods trialed as activity assays for this study, enabling us to rule out food preference as a determinant of observed feeding rates. In the 5 min following the injection, we noted the time (in s) for individuals to start feeding and the total number of feeding forays (with a foray defined as a discrete feeding bout with no interval between bites). Fish that did not feed (*S. doliatus* $n = 5$, *S. lineatus* $n = 2$) were excluded from the analysis of this trait. All behavioral trials (activity level, latency to feed and feeding rate) were scored blind with respect to temperature treatment (LEL).

Statistical analysis

Given the potential for behaviors and somatic traits to be highly correlated within a species, we performed an exploratory linear discriminant analysis (LDA) to examine the level of variation in the dataset explained by species. All measured variables, centered and re-scaled, were included in the analysis with the exception of latency to feed (due to missing values for individuals). The primary axis (LDA1) explained 85.5% of the variation in our trait data, with strong separation of species along this axis. To test for the effects of species and temperature (and their potential interaction) on this variation within the dataset we then used a general linear model with response variable as the factor score of each fish on LDA1, and the species and temperature treatment of each fish as fixed factors in the model. The effect of species (also correlated with body size and age at testing) was significant (GLM, $F_{1,58} = 160.31$, $p < 0.001$), with neither temperature ($F_{2,58} = 1.125$, $p = 0.33$) or the interaction between species and temperature significant ($F_{2,58} = 0.735$, $p = 0.48$). This strong effect of species was demonstrated using predicted probabilities of class membership analysis, where the model was able to assign an individual to the correct species grouping-based 63% of the time. This overwhelming effect of species (or potentially body size or age) on the dataset meant that examination of potential temperature effects across both species would be uninformative. We therefore conducted all further analyses of somatic and behavioral responses within-species in order to determine the effect of

temperature (i.e., thermal sensitivity) on *S. doliatus* and *S. lineatus*.

We ran separate general linear models to test the effect of temperature (fixed factor) on the growth of each species (a) change in standard length, (b) change in mass and (c) change in body depth. In each case, we included the starting value of each growth variable (i.e., starting length, starting mass or starting body depth) as a covariate in the model. To test the effect of temperature on HSI, we ran linear models with individual standard length at the end of the experiment as a covariate. The inclusion of either starting size or end standard length as a covariate was supported by Akaike information criterion (AIC) model comparison (improved model fit with covariate included). Since behavioral metrics did not adhere to normality assumptions of Gaussian linear models, separate generalized linear models were used to examine the effect of temperature on (d) feeding latency (negative binomial error distribution used to correct for over-dispersion), (e) feeding rate (Poisson error distribution with an observation-level random effect of fish ID to account for over-dispersion) and (f) activity level (binomial error distribution). In all the above cases, if the main treatment effect of temperature was significant, we ran Tukey's post-hoc tests to examine pairwise differences between treatments ($p < 0.05$).

To test for the effect of temperature on species' immune responses, we used a two-step analysis. We first ran a series of one-sample t-tests to compare the immune response of each temperature treatment to the null of zero response. Where temperature treatment groups showed a significant immune response we used linear models to check for an effect of temperature (fixed effect) on the level of response. The inclusion of final standard length, weight, and body depth as covariates in these models were explored using AIC model comparisons, and it was determined that they did not significantly improve model fit.

All analyses were performed using *R* and Rstudio (version 1.3.1073) and the associated packages 'ggpubr, 'ggplot2', 'car', 'MuMIn', 'emmeans', 'multcomp', 'lme4', 'candisc', 'caret', 'devtools' and 'AER'.

Results

Starting size and age

At the commencement of the study, juvenile *S. doliatus* were, on average, 2.54 cm standard length (SL) (± 0.03 SE), with an average mass of 0.41 g (± 0.02 SE) and 0.93 cm (± 0.03 SE) body depth. The juvenile *S. lineatus* used in the study were, on average, 6.31 cm SL (± 0.16 SE), with a mass of 7.71 g (± 0.67 SE) and body depth of 2.89 cm (± 0.08 SE). The aging of a subset of individuals showed that *S. doliatus* were between 10 and 50 days post-settlement at the start of the treatment exposure, and thus the individuals used in our study were assumed to be from the Feb and Mar 2019 recruitment events. The aged subset of *S. lineatus* were found to be between 42 and 132 days post-settlement, thus the study individuals were assumed to be from the Dec 2018-Mar 2019 recruitment events.

Growth and development

There was no effect of water temperature on the growth of juvenile *S. doliatus* and *S. lineatus* measured in terms of either increase in standard length (Fig. 1a; *S. doliatus:* mean = 2.7 cm, $F_{2,32} = 2.255$, $p = 0.122$; *S. lineatus*: mean = 1.83 cm, $F_{2,41} = 1.189$, $p = 0.315$), or mass (Fig. 1b; *S. doliatus:* mean = 4.1 g, $F_{2,32} = 0.783$, $p = 0.466$; *S. lineatus*: mean = 9.39 g, $F_{2,41} = 0.768$, $p = 0.471$). We observed a trend of increasing body depth of juvenile *S. doliatus* in elevated water temperature (Fig. 1c; $F_{2,32} = 5.630$, $p = 0.008$), however the changes between temperatures were non-significant (Tukey's post-hoc all $p > 0.05$). There was a significant effect of temperature on the liver to body weight ratio (HSI) of *S. doliatus* (Fig. 1d; $F_{2,29} = 4.077$, $p = 0.027$), with fish held at 31.5 °C in significantly lower HSI than those held at 28.0 °C (Tukey's post-hoc 28.0 vs. 31.5 °C: $p = 0.040$; 30.0 vs. 31.5 °C: $p = 0.059$). By contrast, there was no effect of temperature on either the body depth ($F_{2,41} = 0.732$, $p = 0.487$) or HSI ($F_{2,38} = 0.222$, $i = 0.802$) of juvenile *S. lineatus* (Fig. 1c, d).

Immune response

Both *S. doliatus* ($t_{(35)} = -0.887$, $p = 0.3813$) and *S. lineatus* ($t_{(43)} = 1.6711$, $p = 0.102$) showed no significant response to injection with PBS, validating the use of PHA as a method for measuring immunity in these two species (Fig. 2a). Juvenile *S. doliatus* held at 28.0 and 30.0 °C both mounted a significant immune response in the 24 h following injection with PHA (28.0 °C treatment, $t_{(11)} = 3.048$, $p = 0.011$; 30.0 °C treatment $t_{(12)} = 3.981$, $p = 0.002$), with no difference in the magnitude of response observed at these two temperatures ($t_{(23)} = -0.423$, $p = 0.676$, Fig. 2b). By contrast, *S. doliatus* held at 31.5 °C showed no immune response over the same period ($t_{(10)} = 1.4888$, $p = 0.167$). Juvenile *S. lineatus* were able to mount a significant immune response at all temperature treatments (28.0 °C $t_{(12)} = 2.330$, $p = 0.038$; 30.0 °C $t_{(15)} = 2.268$, $p = 0.039$; 31.5 °C $t_{(15)} = 2.629$, $p = 0.019$) (Fig. 2b), with no difference in the magnitude of this response across temperatures ($F_{2,41} = 0.016$, $p = 0.984$).

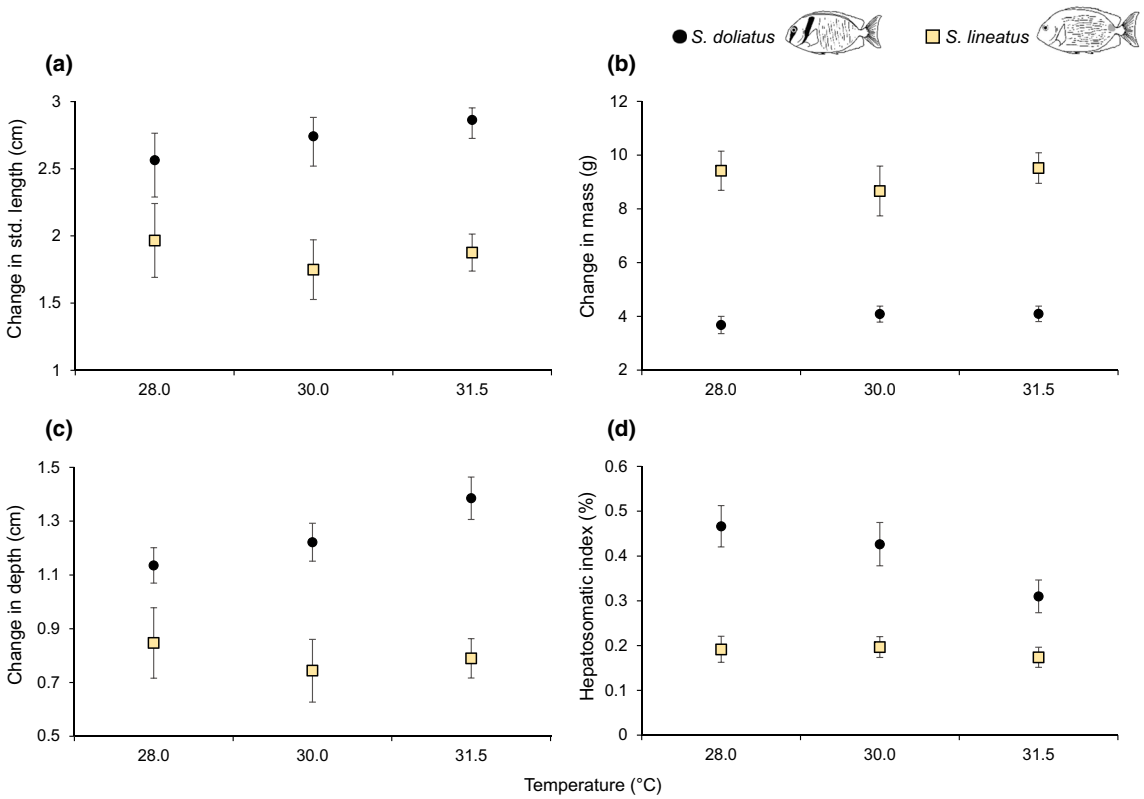

Fig. 1 Change in **a** standard length, **b** body mass, **c** body depth and **d** hepatosomatic index (HSI) of *S. doliatus* (n = 12, 13, 11) (28, 30, 31.5 °C) and *S. lineatus* (n = 13, 16, 16) (28, 30, 31.5 °C). In all cases, data are means ± SE

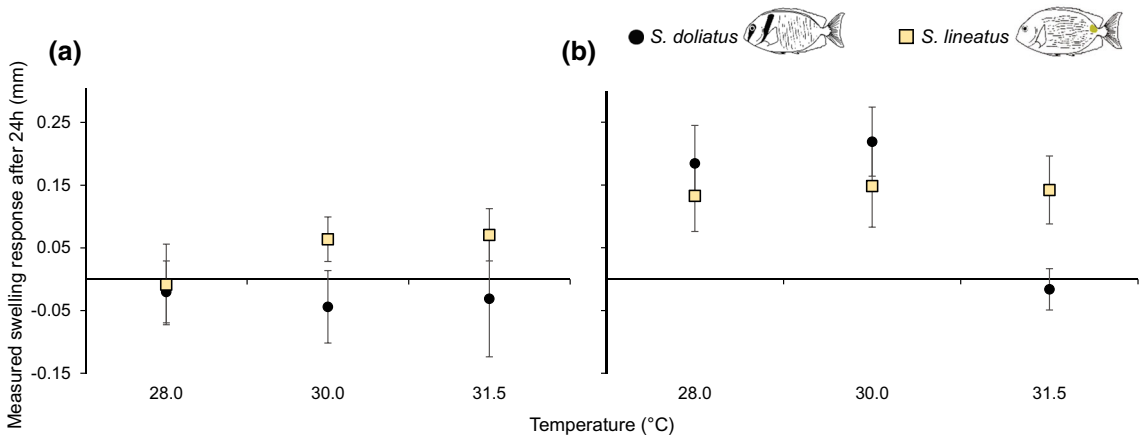

Fig. 2 Immune response of *S. doliatus* and *S. lineatus* measured as change in caudal peduncle lateral width thickness over a 24 h period. of *S. doliatus* (n = 12, 13, 8) and *S. lineatus* (n = 13, 16, 16), following **a** a control injection with phosphate buffered saline (PBS) solution and **b** a phytohaemagglutinin (PHA) injection. Data are means ± SE

Behavior

Individuals of both species generally fed within the first 45 s of food being provided. For *S. doliatus* there was no effect of temperature on latency to feed (Fig. 3a; GLM, $\chi^2_{(2)}$ = 2.621 p = 0.270), with fish at the ambient temperature visually exhibiting greater variation in their feeding latency than individuals at both of the higher temperatures although this was not statistically supported (Fligner Killeen test, $\chi^2_{(2)}$ = 1.127, p = 0.57). *S. lineatus* fed faster in warmer temperature treatments (Fig. 3a; GLM, $\chi^2_{(2)}$ = 13.503, p = 0.001) with no difference in variation observed between temperature groups (Fligner Killeen test, $\chi^2_{(2)}$ = 5.006, p = 0.08). The reduction in latency to feed by *S. lineatus* was significant when comparing fish held in

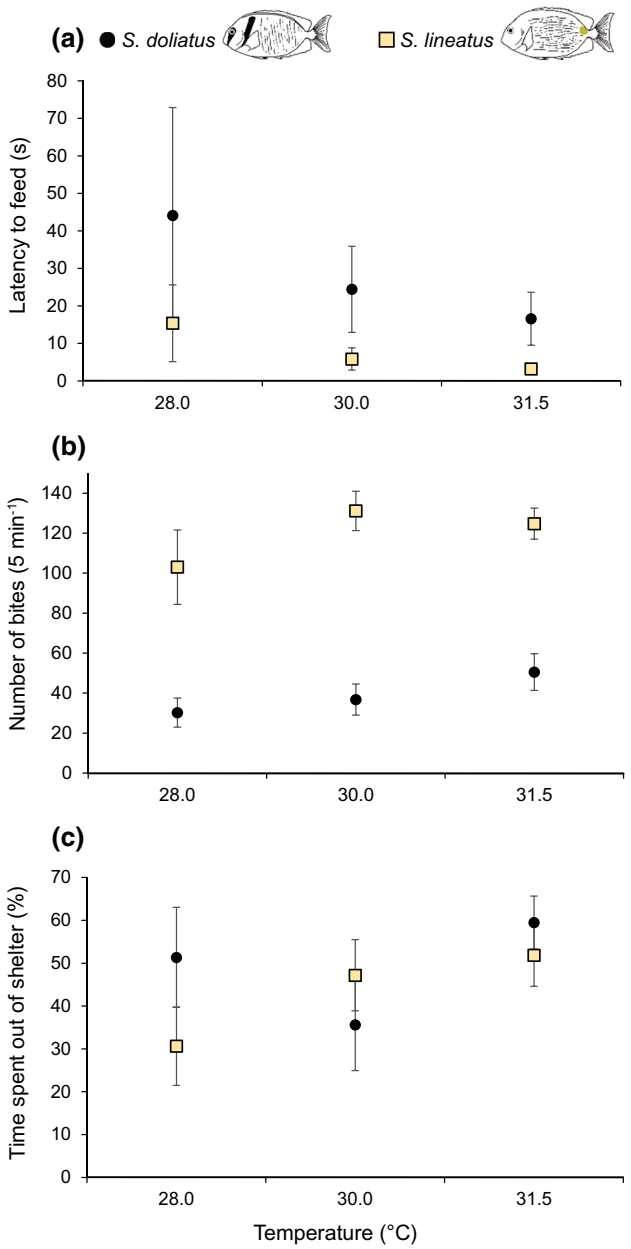

Fig. 3 Behavioral traits of juvenile *S. doliatus* and *S. lineatus* at three experimental temperature treatments: **a** time until first feeding (i.e., latency to feed) of *S. doliatus* (n = 10, 10, 9) and *S. lineatus* (n = 11, 16, 16); **b** number of bites taken within 5 min by *S. doliatus* (n = 12, 13, 10) and *S. lineatus* (n = 11, 16, 16); (c) time spent out of shelter by *S. doliatus* (n = 12, 13, 11) and *S. lineatus* (n = 13, 16, 16). In all cases, data are means ± SE

the 31.5 °C treatment to those held at 28.0 °C (Tukey's, p = 0.001).

For both species, feeding rates increased with increasing water temperature (Fig. 3b), although for *S. doliatus* there was no statistical effect of temperature on feeding rate (Fig. 3b; GLM, $\chi^2_{(2)}$ = 1.785, p = 0.410). *S. lineatus* feeding rate was significantly greater in the higher temperature treatments (Fig. 3b; GLM, $\chi^2_{(2)}$ = 6.183, p = 0.045) driven by differences between individuals at 28.0 °C and both 30.0 °C (z = 2.26, p = 0.024) and 31.5 °C (z = 2.14, p = 0.033).

There was no effect of temperature on the activity level of *S. doliatus* (GLM, $\chi^2_{(2)}$ = 1.45, p = 0.484) or *S. lineatus* (GLM, $\chi^2_{(2)}$ = 1.44, p = 0.486), however *S. lineatus* displayed a more consistent general increase in activity levels with increasing temperature (Fig. 3c). Individuals of this species in the 30.0 and 31.5 °C treatments were, on average, 19% more active than those housed at 28.0 °C (Fig. 3c).

Discussion

Previous studies of effects of increasing ocean temperature on the growth and physiological condition of coral reef fishes have focused on small, site-attached species, such as damselfish. Here we investigated the thermal sensitivity of two closely-related, but habitat-distinct species of mobile fishes belonging to the family Siganidae, following a period of a 14 week exposure to ambient and elevated temperatures during juvenile development. In comparison to results previously reported for site-attached species, which displayed high thermal sensitivity and negative physical effects at 1.5 to 3 °C warming above summer averages (Munday et al. 2008b; Zarco-Perelló et al. 2012; Motson and Donelson 2017), we found evidence of robustness of juvenile rabbitfish to elevated thermal conditions. Although juvenile *S. lineatus* exhibited behavioral changes (feeding rates and propensity to feed) in response to higher water temperatures, they were able to maintain growth, physical condition and immune function across the 28.0 to 31.5 °C developmental temperature range. Juvenile *S. doliatus* did show greater sensitivity in terms of physiological response, with significant reductions in HSI and immuno-competence at 31.5 °C. The observed differences between the two species are most likely linked to adaptations to the different habitats with which they are associated, with *S. lineatus* primarily associated with estuarine and mangrove habitats that experience greater thermal variation daily and seasonally (Kenny 1974; Wolanksi et al. 1981; Whitfield 1999; DAFF 2018). Observed differences may, of course, also be partially attributable to the effects of the size difference between the species (*S. lineatus* individuals being larger than *S. doliatus*) and the slight differences in their ages when tested.

Overall, our results suggest that juvenile rabbitfish may be less sensitive to small increases in water temperature than previously studied site-attached species such as damselfish, cardinalfish and wrasses (Sponaugle et al. 2006; Munday et al. 2008a, b; Gardiner et al. 2010; Nilsson et al. 2010; Nowicki et al. 2012; Zarco-Perelló et al. 2012;

Motson and Donelson 2017). Juvenile rabbitfish in the current study also exhibited lower thermal sensitivity than larval and adult coral trout (*Plectropomus spp.*) in terms of development and growth, although they did demonstrate similar increases in feeding rate at elevated temperatures (Pratchett et al. 2013; Johansen et al. 2015). The absence of significant observed physiological effects for *S. lineatus* and *S. doliatus* at 30.0 °C and *S. lineatus* at 31.5 °C is encouraging, given that both species, as well as the family more broadly, provide essential ecosystem services on Indo-Pacific coral reefs (Fox and Bellwood 2008, 2013; Fox et al. 2009; Hoey et al. 2013) and are targets of commercial and artisanal food fisheries in many parts of the Indo-Pacific (Campos et al. 1994; Rhodes et al. 2008; Soliman et al. 2009; Soliman and Yamaoka 2010; Gillett 2011). It is perhaps also worth noting here that, with respect to behavior and immune response in *S. doliatus* we recorded considerable levels of variation between individuals, suggesting that even within the relatively small number of individuals sampled for the current experiment, there exists intraspecific variation. This variation could potentially provide a target for selection processes to act upon during warming events, suggesting a potential role for evolutionary responses to rising temperatures.

Traits in juvenile *S. doliatus* that were affected by water temperature were related to physiological stress responses and were seen only in the group exposed to 31.5 °C. Populations of *S. doliatus* on the Great Barrier Reef, Australia already experience temperatures of 31.5 °C during heatwave events associated with thermal anomalies (Hughes et al. 2017, 2018; Eakin et al. 2019), although only for periods of days. Physical condition (HSI) and immunity were significantly reduced when fish developed at 31.5 °C, both of which could impact negatively on individual survival in a natural setting (Wendelaar Bonga 1997; Barton 2002; Stallings et al. 2010). If prolonged, the physiological effects of thermal stress could therefore lead to changes in whole organism attributes such as growth and behavior (Wedemeyer and McLeay 1981; Wendelaar Bonga 1997; Barton 2002). While we observed trends of decreased latency and increased feeding rate with warming, *S. doliatus* showed no significant differences in behavior between temperature treatments. We also observed no differences in the overall length and mass of fish exposed to higher temperatures, although fish had greater body depth as water temperature increased (counter to the expected pattern of reduced growth at elevated temperatures (Munday et al. 2008b; Zarco-Perelló et al. 2012; Motson and Donelson 2017)). This result could have been due to the experimental temperatures being within the thermal tolerance of the species, meaning that higher temperature treatments could have simply provided a thermal advantage in terms of growth rate. It is also possible that the absence of length and mass effects of temperature in *S. doliatus* (and in *S. lineatus*) were due to entrainment of ontogenetic growth patterns that are exhibited by many juvenile reef fish as a means of avoiding predation at smaller body sizes (Werner and Hall 1988; Dahlgren and Eggleston 2000; Persson et al. 2000). If so, this could suggest that the negative effects of temperature on HSI and immune function observed in *S. doliatus* were the result of energetic trade-offs, with resources diverted to maintenance of growth, at the expense of immunity and stored energy (Werner and Hall 1988; Angilletta et al. 2003; Clotfelter et al. 2007; Sandersfeld et al. 2015). Finally, the absence of any temperature effect on measured growth and behavioral traits in juvenile *S. doliatus* could also be a temporal phenomenon, with effects of sustained exposure to elevated temperatures in early life only becoming visible much later in life in terms of impact on adult life-history (Monaghan 2008; Jonsson and Jonsson 2014). For example, exposure to poor environmental conditions during development may have negative effects on adult growth, reproduction and rate of aging (Nussey et al. 2007; Marshall et al. 2017; Cooper and Kruuk 2018) in a reversal of 'silver spoon' hypothesis (Grafen 1990). The current study did not take into account these potential longer-term trade-offs representing a critical future research priority.

Juvenile *S. lineatus* were robust to exposure to temperatures from 28.0 to 31.5 °C in terms of their growth and physiology (body length, body depth, mass, physical condition and immunity were not significantly affected). However, latency to feed decreased and feeding rate increased with warming potentially indicating a greater need to acquire more energy at higher temperatures. While not measured directly in this study, elevated water temperature increases basal metabolic costs in fish (Pörtner 2001; Pörtner and Farrell 2008; Norin et al. 2014; Hoey et al. 2016). It is therefore likely that the increased propensity to feed by *S. lineatus* was driven by a need to meet additional energetic costs at warmer temperatures and maintain growth and condition across the temperature range (Brett 1979; Bowen et al. 1995; Arrington et al. 2002; Sandersfeld et al. 2015). The lack of any temperature effect on HSI and immune function in this species (contrary to *S. doliatus*) supported our hypothesis that juvenile *S. lineatus* would exhibit higher thermal tolerance, due to their association with estuarine habitats. However, the absence of any perceived costs of the higher energetic demands of warmer temperatures in *S. lineatus* could also have been partially due to their larger size at testing than *S. doliatus*, or could be an artifact of laboratory conditions, under which fish were fed ad libitum twice daily and are therefore likely to have access to ample compensatory food resources. In the wild, this may or may not be the case

(Clements et al. 2009) and could also be contingent on the productivity of reef and estuarine ecosystems under future climate conditions, where studies have shown the potential for reductions in primary production under global change (Johnson et al. 2017; Graba-Landry et al. 2020).

S. doliatus and *S. lineatus* are known to share a close phylogenetic relationship (Kuriiwa et al 2007; Borsa et al. 2007; Siqueira et al. 2019) with the two species consistently positioned within the same phylogenetic clade having diverged from a common ancestor only about 2½ million years ago (Siqueira et al. 2019). However, they are primarily associated with two different habitat types, *S. doliatus* being strongly reef-associated and *S. lineatus* typically associated with estuarine and mangrove habitats (although it does also occur on reefs with neighboring mangrove areas). Is it possible, then, to extrapolate physiological and behavioral responses to thermal stress between sister congeners from different habitats? Our study suggests not: *S. doliatus and S. lineatus* showed differences in (1) the nature and (2) the threshold of their temperature responses. *S. lineatus* exhibited behavioral changes while *S. doliatus* exhibited a physiological response with decreased immunity and physical condition with warming. As we outline above, these differences are likely attributable to adaptations associated with their different habitats, with *S. lineatus* being adapted to estuarine environments. Since *S. lineatus* also has a broader habitat niche (being associated with areas of coral reef as well as shallow mangroves and estuaries), the species might be expected to have a broader range of physiological tolerances than the coral reef specialist, *S. doliatus*.

The second notable difference in the responses exhibited by these two species was the temperature at which we observed the respective effects. For *S. doliatus,* effects were seen only at the highest temperature treatment group (31.5 °C), while for *S. lineatus* we recorded effects even at 30.0 °C. The lower temperature threshold for observation of behavioral changes could be due to the lower relative costs of adopting the strategy of behavioral plasticity in response to the environmental variation (*S. lineatus*) compared to those associated with making physiological changes (*S. doliatus*) (Clark et al. 2013; Wong and Candolin 2015; Habary et al. 2017). It is also possible that *S. lineatus* juveniles were more readily able to show a positive behavioral response at 30.0 °C and 31.5 °C due to their prior exposure to similar fluctuations within their natural estuarine setting (Brett 1979; Bowen et al. 1995; Arrington et al. 2002; Sandersfeld et al. 2015). The differences in thermal sensitivity observed in the current study for these two species could suggest that they have different thermal performance ranges, based on their different habitats. Certainly previous studies from freshwater environments have demonstrated that thermal history associated with habitat or geographic cline can be a driver of variation in thermal sensitivity even within populations of a single species (Chrétien and Chapman 2016; Reizenberg et al. 2018), and the fact that changes in ocean temperature can influence the selection of different habitats by species (Freitas et al. 2016). To our knowledge, however, there have be no previous investigations of inter-habitat differences in the thermal sensitivity of closely-related, reef-associated species. The negative physiological effects observed for juvenile *S. doliatus* at 31.5 °C suggests to us that this temperature lies above the thermal optimum for this species, whereas for juvenile *S. lineatus* our treatments did not exceed its optimal thermal range. As noted above however, thermal effects on the juvenile life-stage cannot necessarily be extrapolated to adult organisms when determining species' thermal performance range, temperatures conducive to early growth and development can impact negatively on later life stages (e.g., effects on maturity timing, reproductive capacity) (Munday et al. 2008a; Hofmann and Todgham 2010; Donelson et al. 2011; Pankhurst and Munday 2011).

Our study examined the thermal sensitivity of two closely-related yet habitat-distinct species of mobile reef fishes belonging to an under-studied functional group (rabbitfish). By broadening the study of thermal tolerance of reef fishes beyond its current focus on site-attached species such as damselfish (Pomacentridae) (Munday et al. 2008a, b; Nowicki et al. 2012; Zarco-Perelló et al. 2012), wrasses (Labridae) (Sponaugle et al. 2006; Motson and Donelson 2017) and cardinalfish (Apogonidae) (Gardiner et al. 2010; Nilsson et al. 2010), our results make a novel contribution to the understanding of how temperature increases expected under future climate change will impact critical ecosystem services (herbivory and detritivory) along with human food security (fisheries catches). Our study showed that juvenile *S. doliatus* are thermally sensitive in terms of their physiology while *S. lineatus* respond to increased temperature via changes in their feeding behavior. We found that, under warmer conditions, *S. lineatus* increased their feeding rate while maintaining growth and physical condition. Whether this behavioral buffering could occur in a natural setting would depend on availability of food resources. Interestingly, both species showed lower thermal sensitivity than has been recorded previously for site-attached and mobile species, highlighting that not all reef fish species show equal responses to temperature change. This is certainly encouraging, especially considering that there is also the potential for these mobile species to further buffer the effects of environmental change via movement and behavioral thermoregulation (not accounted for in the current laboratory setting). Incorporating this aspect of ecological realism into our studies via observations of field-active individuals remains

a key research priority. While our results suggest that juvenile rabbitfish may be robust to low levels of future global warming, it will also be important to investigate larval and reproductive stages, as these have been shown to be the most sensitive and likely to limit survival in a warmer future ocean (Houde 1989; Green and Fisher 2004; Pankhurst and Munday 2011; Munday et al. 2013; McLeod et al. 2013; Donelson et al. 2010, 2016; Hoey et al. 2016). Understanding the mechanistic drivers of our findings (undetermined in the current study) via the measurement of cellular- or tissue-level physiological parameters (e.g., enzyme activities, hematology, metabolomics), as well as testing additional (or fluctuating) temperature regimes to fully describe the thermal performance curves of the measured traits in these rabbitfish species would represent useful avenues for future research. Finally, our findings suggest there are likely to be inaccuracies associated with extrapolating physiological and behavioral responses across closely-related species associated with different reef habitats. Our results therefore point to a strong role of habitat in determining the thermal sensitivity of individual reef fish species.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Angilletta Jr MJ, Wilson RS, Navas CA, James RS (2003) Tradeoffs and the evolution of thermal reaction norms. Trends Ecol Evol 18:234-240

Arrington DA, Winemiller KO, Loftus WF, Akin S (2002) How often do fishes "run on empty"? Ecology 83:2145-2151

Australian Institute of Marine Science (AIMS) (2017) AIMS sea water temperature observing system (AIMS temperature logger program) https://doi.org/10.25845/5b4eb0f9bb848

Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol 42:517-525

Bellwood DR, Hughes TP, Folke C, Nyström M (2004) Confronting the coral reef crisis. Nature, 429:827

Bennett S, Duarte CM, Marbà N, Wernberg T (2019) Integrating within-species variation in thermal physiology into climate change ecology. Phil Trans Roy Soc B 374: 20180550

Bernal MA, Schunter C, Lehmann R, Lightfoot DJ, Allan BJ, Veilleux HD, Rummer JL, Munday PL, Ravasi T (2020) Species-specific molecular responses of wild coral reef fishes during a marine heatwave. Sci Adv 6:3423

Borsa P, Lemer S, Aurelle D (2007) Patterns of lineage diversification in rabbitfishes. Mol Phylogenet Evol 44:427-435

Bowen SH, Lutz EV, Ahlgren MO (1995) Dietary protein and energy as determinants of food quality: trophic strategies compared. Ecology 76:899-907

Boyles JG, Seebacher F, Smit B, McKechnie AE (2011) Adaptive thermoregulation in endotherms may alter responses to climate change. Integr Comp Biol 51:676-690

Brett JR (1979) Environmental factors and growth. In: Fish physiology vol III: bioenergetics and growth, WS Hoar, DJ Randall and JR Brett (eds) pp. 599–675

Campos W, Del Norte-Campos A, McManus J (1994) Yield estimates, catch, effort and fishery potential of the reef flat in Cape Bolinao, Phillipines. J Appl Ichthyol 10: 82–95

Castillo-Vargasmachuca S, Ponce-Palafox J, Arambul-Muñoz E, Lopez-Gomez C, Arredondo-Figueroa JL, Spanopoulos-Hernandez M (2017) The combined effects of salinity and temperature on the proximate composition and energetic value of spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869). Lat Am J Aquat Res 45:1054-1058

Cheal AJ, MacNeil MA, Cripps E, Emslie MJ, Jonker M, Schaffelke B, Sweatman H (2010) Coral–macroalgal phase shifts or reef resilience: links with diversity and functional roles of herbivorous fishes on the Great Barrier Reef. Coral reefs 29:1005-1015

Childs AR, Cowley PD Næsje TF, Booth AJ, Potts WM, Thorstad EB, Økland F (2008) Do environmental factors influence the movement of estuarine fish? A case study using acoustic telemetry. Estuar Coast Shelf S 78:227–236

Chrétien E, Chapman LJ (2016) Tropical fish in a warming world: thermal tolerance of Nile perch *Lates niloticus* (L.) in Lake Nabugabo, Uganda. Conserv Phys 4 https://doi.org/10.1093/conphys/cow062

Clark TD, Messmer V, Tobin AJ, Hoey AS and Pratchett MS (2017) Rising temperatures may drive fishing-induced selection of low-performance phenotypes. Sci Rep 7:1-11

Clark TD, Sandblom E, Jutfelt F (2013) Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J Exp Biol 216:2771-2782

Clements KD, Raubenheimer D, Choat JH (2009) Nutritional ecology of marine herbivorous fishes: ten years on. Funct Ecol 23:79-92

Clotfelter ED, Ardia DR, McGraw KJ (2007) Red fish, blue fish: trade-offs between pigmentation and immunity in *Betta splendens*. Behav Ecol 18:1139-1145

Cooper EB, Kruuk LE (2018) Ageing with a silver-spoon: A meta-analysis of the effect of developmental environment on senescence. Evol Lett 2:460-471

Crozier LG, Hutchings JA (2014) Plastic and evolutionary responses to climate change in fish. Evol Appl 7:68-87

Dahlgren CP, Eggleston DB (2000) Ecological processes underlying ontogenetic habitat shifts in a coral reef fish. Ecology 81:2227-2240

Department of Agriculture, Fisheries, and Forestry (DAFF), Queensland Government (2018) Ambient estuarine water quality monitoring data – 2012 to present day. https://www.data.qld.gov.au/dataset/ambient-estuarine-water-quality-monitoring-data-near-real-time-sites-2012-to-present-day

Dittmar J, Janssen H, Kuske A, Kurtz J, Scharsack JP (2014) Heat and immunity: an experimental heat wave alters immune functions in three-spined stickleback (*Gasterosteus aculeatus*). J Anim Ecol 83:744-757.

Donelson JM, Munday PL, McCormick MI, Nilsson GE (2011) Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. Glob Chang Biol 17:1712-1719

Donelson JM, Munday PL, McCormick MI, Pankhurst NW, Pankhurst PM (2010) Effects of elevated water temperature and food availability on the reproductive performance of a coral reef fish. Mar Eco Prog Ser 401:233-243

Donelson JM, Wong M, Booth DJ, Munday PL (2016) Transgenerational plasticity of reproduction depends on rate of warming across generations. Evol Appl 9:1072-81

Eakin, CM, Sweatman, HP, Brainard, RE (2019) The 2014–2017 global-scale coral bleaching event: insights and impacts. Coral Reefs 38:539-545

Freitas C, Olsen EM, Knutsen H, Albretsen J, Moland E (2016) Temperature-associated habitat selection in a cold-water marine fish. J Anim Ecol 85: 628-637

Fox RJ, Bellwood DR (2008) Remote video bioassays reveal the potential feeding impact of the rabbitfish *Siganus canaliculatus* (f: Siganidae) on an inner-shelf reef of the Great Barrier Reef. Coral Reefs 27:605-615

Fox RJ, Bellwood DR (2013) Niche partitioning of feeding microhabitats produces a unique function for herbivorous rabbitfishes (Perciformes, Siganidae) on coral reefs. Coral Reefs 32:13-23

Fox RJ, Bellwood DR (2011) Unconstrained by the clock? Plasticity of diel activity rhythm in a tropical reef fish, Siganus lineatus. Funct Ecol 25:1096-1105

Fox RJ, Sunderland TL, Hoey AS, Bellwood DR (2009) Estimating ecosystem function: contrasting roles of closely related herbivorous rabbitfishes (Siganidae) on coral reefs. Mar Eco Prog Ser 385:261-269

Gardiner NM, Munday PL, Nilsson GE (2010) Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. PloS one 5:13299

Gillett R (2011) Fisheries of the Pacific Islands: regional and national information. FAO

Graba-Landry AC, Loffler Z, McClure EC, Pratchett MS, Hoey AS (2020) Impaired growth and survival of tropical macroalagae (*Sargassum* spp.) at elevated temperatures. Coral Reefs 39:475-486

Grafen A (1990) Biological signals as handicaps. J Theor Biol 144:517-546

Graham NA, Jennings S, MacNeil MA, Mouillot, D, Wilson SK (2015) Predicting climate-driven regime shifts versus rebound potential in coral reefs. Nature 518:94-97

Great Barrier Reef Marine Park Authority (GBRMPA) (2019) Great Barrier Reef Outlook Report 2019. Great Barrier Reef Marine Park Authority

Green BS, Fisher R (2004) Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. J Exp Mar Biol Ecol 299:115-132

Habary A, Johansen JL, Nay TJ, Steffensen JF, Rummer JL (2017) Adapt, move or die–how will tropical coral reef fishes cope with ocean warming?. Glob Chang Biol 23:566-577

Harrison TD, Whitfield AK (2006) Application of a multimetric fish index to assess the environmental condition of South African estuaries. Estuar Coast 29:1108-1120

Hobday AJ, Lough JM (2011) Projected climate change in Australian marine and freshwater environments. Mar Freshwater Res 62:1000-1014

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N. (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742

Hoey AS, Brandl SJ, Bellwood, DR (2013) Diet and cross-shelf distribution of rabbitfishes (f. Siganidae) on the northern Great Barrier Reef: implications for ecosystem function. Coral Reefs 32:973-984

Hoey AS, Howells E, Johansen JL, Hobbs JPA, Messmer V, McCowan DM, Wilson SK, Pratchett MS (2016) Recent advances in understanding the effects of climate change on coral reefs. Diversity 8:12

Hofmann GE, Todgham AE (2010) Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Annu Rev Physiol 72:127-145

Houde ED (1989) Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fish B-NOAA 87:471-495

Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM, Baird AH, Baum JK, Berumen ML, Bridge TC, Claar DC (2018) Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science 359:80-83

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JB, Kleypas J, Lough JM (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929-933

Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, Babcock RC, Beger M, Bellwood DR, Berkelmans R, Bridge TC (2017) Global warming and recurrent mass bleaching of corals. Nature 543:373

Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett MS, Steneck RS, Willis B (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. Curr Biol 17:360-365

Iglesias-Carrasco M, Fox RJ, Vega-Trejo R, Jennions MD, Head ML (2019a) An experimental test for body size-dependent effects of male harassment and an elevated copulation rate on female lifetime fecundity and offspring performance. J Evol Biol 32:1262-1273

Iglesias-Carrasco M, Fox RJ, Vincent A, Head ML, Jennions MD (2019b) No evidence that male sexual experience increases mating success in a coercive mating system. Anim Behav 150:201-208

Intergovernmental Panel on Climate Change (IPCC) (2019) Special report on the ocean and cryosphere in a changing climate (SROCC). M Barange, B Seibel (eds) Geneva, Switzerland

Johansen JL, Jones GP (2011) Increasing ocean temperature reduces the metabolic performance and swimming ability of coral reef damselfishes. Global Change Biol 17:2971-2979

Johansen JL, Pratchett MS, Messmer V, Coker DJ, Tobin AJ, Hoey AS (2015) Large predatory coral trout species unlikely to meet increasing energetic demands in a warming ocean. Sci Rep 5:13830

Johnson MD, Comeau S, Lantz CA Smith JE (2017) Complex and interactive effects of ocean acidification and temperature on epilithic and endolithic coral-reef turf algal assemblages. Coral Reefs 36:1059–1070

Jonsson B, Jonsson N (2014) Early environment influences later performance in fishes. J Fish Biol 85:151-188

Kenny R, (1974) Inshore surface sea temperatures at Townsville. Mar Freshwater Res 25:1-5

King AD, Karoly DJ, Henley BJ (2017) Australian climate extremes at 1.5°C and 2°C of global warming. Nat Clim Change 7:412

Kuronuma K (1961) A checklist of fishes of Vietnam. United States Consultants, Inc.; International Cooperation Administration Contract-IV-153. Division of Agriculture and Natural Resources, United States Operations Mission to Vietnam. pp. 66

Kuiter RH, Tonozuka T (2001) Pictorial guide to Indonesian reef fishes. Part 3. Jawfishes - Sunfishes, Opistognathidae - Molidae. Genetics, Australia. pp. 623–893

Kuriiwa K, Hanzawa N, Yoshino T, Kimura S, Nishida M (2007) Phylogenetic relationships and natural hybridization in

rabbitfishes (Teleostei: Siganidae) inferred from mitochondrial and nuclear DNA analyses. Mol Phylogenet Evol 45:69-80

Larios-Soriano E, Re-Araujo AD, Díaz F, Sánchez CG, López-Galindo L, Castro LI, Ramírez DT (2020) Effect of acclimation temperature on thermoregulatory behaviour, thermal tolerance and respiratory metabolism of *Lutjanus guttatus* and the response of heat shock protein 70 (Hsp70) and lactate dehydrogenase (Ldh-a) genes. Aquac Res 51:1089-1100

Lieske E, Myers R (2002) Coral reef fishes: Indo-pacific and Caribbean (No. Sirsi) i9780691089959)

Madeira D, Narciso L, Cabral HN, Vinagre C, Diniz MS (2013) Influence of temperature in thermal and oxidative stress responses in estuarine fish. Comp Biochem Phys A 166:237-243

Marshall HH, Vitikainen EI, Mwanguhya F, Businge R, Kyabulima S, Hares MC, Inzani E, Kalema-Zikusoka G, Mwesige K, Nichols HJ, Sanderson JL (2017) Lifetime fitness consequences of early-life ecological hardship in a wild mammal population. Ecol Evol 7:1712-1724

Mazumder SK, Abd Ghaffar M, Das SK (2020) Effect of temperature and diet on gastrointestinal evacuation of juvenile malabar blood snapper (*Lutjanus malabaricus* Bloch & Schneider, 1801). Aquaculture 522:735114

McLeod IM, Rummer JL, Clark TD, Jones GP, McCormick MI, Wenger AS, Munday PL (2013) Climate change and the performance of larval coral reef fishes: the interaction between temperature and food availability. Conserv Physiol 1

Monaghan P (2008) Early growth conditions, phenotypic development and environmental change. Philos T R Soc B 363:1635-1645

Motson K, Donelson JM (2017) Limited capacity for developmental thermal acclimation in three tropical wrasses. Coral Reefs 36:609-621

Munday PL (2014) Transgenerational acclimation of fishes to climate change and ocean acidification. F1000Prime Rep 6

Munday PL, Donelson JM, Dixson DL, Endo GG (2009) Effects of ocean acidification on the early life history of a tropical marine fish. P Roy Soc B-Biol Sci 276:3275-3283

Munday PL, Jones GP, Pratchett MS, Williams AJ (2008a) Climate change and the future for coral reef fishes. Fish Fish 9:261-28

Munday PL, Kingsford MJ, O'Callaghan M, Donelson JM (2008b) Elevated temperature restricts growth potential of the coral reef fish *Acanthochromis polyacanthus*. Coral Reefs 27:927-931

Munday PL, Warner RR, Monro K, Pandolfi JM, Marshall DJ (2013) Predicting evolutionary responses to climate change in the sea. Ecol Lett 16:1488-1500

Nilsson GE, Östlund-Nilsson S, Munday, PL (2010) Effects of elevated temperature on coral reef fishes: loss of hypoxia tolerance and inability to acclimate. Comp Biochem Phys A 156:389-393

Nowicki JP, Miller GM, Munday PL (2012) Interactive effects of elevated temperature and CO^2 on foraging behavior of juvenile coral reef fish. J Exp Mar Biol Ecol 412:46-51

Norin T, Malte H, Clark TD (2014) Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. J Exp Biol 217:244-251

Nussey DH, Kruuk LE, Morris A, Clutton-Brock TH (2007) Environmental conditions in early life influence ageing rates in a wild population of red deer. Curr Biol 17:1000-1001

O'Connor CM, Reddon AR, Marsh-Rollo SE, Hellmann JK, Ligocki IY, Hamilton IM, Balshine S (2014) A comparative study of an innate immune response in Lamprologine cichlid fishes. Naturewissenschaften 101:839-849

Pankhurst NW, Munday PL (2011) Effects of climate change on fish reproduction and early life history stages. Mar Freshwater Res 62:1015-26

Parmesan C, Root TL, Willig MR (2000) Impacts of extreme weather and climate on terrestrial biota. B Am Meteorol Soc 81:443-450

Persson L, Byström P, Wahlström E, Nijlunsing A, Rosema S (2000) Resource limitation during early ontogeny: constraints induced by growth capacity in larval and juvenile fish. Oecologia 122:459-469

Petitjean Q, Jean S, Côte J, Lamarins A, Lefranc M, Santos R, Perrault A, Laffaille P, Jacquim L (2020) Combined effects of temperature increase and immune challenge in two wild gudgeon populations. Fish Physiol Biochem 46:157-176

Pinsky ML, Eikeset AM, McCauley DJ, Payne JL, Sunday JM (2019) Greater vulnerability to warming of marine versus terrestrial ectotherms. Nature 569:108

Pörtner H (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88:137-146

Pörtner HO, Farrell AP (2008) Physiology and climate change. Science 322: 690-692

Pratchett MS Hoey AS, Wilson SK, Messmer V, Graham NA (2011) Changes in biodiversity and functioning of reef fish assemblages following coral bleaching and coral loss. Diversity 3:424-452

Prachett MS, Messmer V, Reynolds A, Martin J, Clark TD, Munday PL, Tobin AJ, Hoey AS (2013) Effects of climate change on reproduction, larval development, and adult health of coral trout (*Plectropomus* spp.)

Rasher DB, Hoey AS, Hay ME (2013) Consumer diversity interacts with prey defenses to drive ecosystem function. Ecology 94:1347-1358

Reizenberg J-L, Bloy LE, Wely OLF, Shelton JM, Dallas HF (2018) Variation in thermal tolerances of native freshwater fishes in South Africa's Cape Fold Ecoregion: examining the east-west gradient in species' sensitivity to climate warming. J Fish Biol 94: 103-112

Rhodes KL, Tupper MH, Wichilmel CB (2008) Characterization and management of the commercial sector of the Pohnpei coral reef fishery, Micronesia. Coral Reefs 27:443–454

Rodgers GG, Donelson JM, McCormick MI, Munday PL (2018) In hot water: sustained ocean warming reduces survival of a low-latitude coral reef fish. Mar Biol 165:73

Rummer, JL, Couturier, CS, Stecyk, JA, Gardiner, NM, Kinch, JP, Nilsson, GE and Munday, PL (2014) Life on the edge: thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. Global Change Biol 20:1055-1066

Sabapathy U, Teo LH (1994) Some kinetic properties of amylase from the intestine of the rabbitfish, *Siganus canaliculatus* (Park). Comp Biochem Phys B 109:139-144

Saoud IP, Mohanna C, Ghanawi J (2008) Effects of temperature on survival and growth of juvenile spinefoot rabbitfish (*Siganus rivulatus*). Aquac Res 39:491-497

Sandersfeld T, Davison W, Lamare MD, Knust R, Richter C (2015) Elevated temperature causes metabolic trade-offs at the whole-organism level in the Antarctic fish *Trematomus bernacchii*. J exp Biol 218:2373-2381

Siqueira AC, Bellwood DR, Cowman PF (2019) The evolution of traits and functions in herbivorous coral reef fishes through space and time. Proc Roy Soc B 286:20182672

Soliman VS, Bobiles RU, Yamaoka K (2009) Overfishing of three siganid species (Family: Siganidae) in Lagonoy Gulf , Philippines. Kuroshio Science 2:145–150

Soliman VS, Yamaoka K (2010) Assessment of the fishery of siganid juveniles caught by bagnet in Lagonoy Gulf, Southeastern Luzon, Philippines. J Appl Ichthyol 26:561-567

Sponaugle S, Grorud-Colvert K, Pinkard D (2006) Temperature-mediated variation in early life history traits and recruitment success of the coral reef fish Thalassoma bifasciatum in the Florida Keys. Mar Ecol Prog Ser 308:1-15

Stallings CD, Coleman FC, Koenig CC, Markiewicz DA (2010) Energy allocation in juveniles of a warm-temperate reef fish. Environ. Biol. 88:389-98

Sunday JM, Bates, AE, Dulvy NK (2011) Global analysis of thermal tolerance and latitude in ectotherms. P Roy Soc B-Biol Sci 278:1823-1830

Tang S, Graba-Landry A, Hoey AS (2020) Density and height of *Sargassum* influence rabbitfish (f. Siganidae) settlement on inshore reef flats of the Great Barrier Reef. Coral Reefs 39:1-7

Taylor B, Gourley J, Trianni M (2017) Age, growth, reproductive biology and spawning periodicity of the forktail rabbitfish (*Siganus argenteus*) from the Mariana Islands. Mar Freshw Res 68: 1088-1097

Tewksbury JJ, Huey RB, Deutsch CA (2008) Putting the heat on tropical animals. Science 320:1296-1297

Veilleux HD, Ryu T, Donelson JM, Munday PL, Ravasi T (2018) Molecular response to extreme summer temperatures differs between two genetically differentiated populations of a coral reef fish. Front Mar Sci 5:349

Waltham NJ, Sheaves M (2017) Acute thermal tolerance of tropical estuarine fish occupying a man-made tidal lake, and increased exposure risk with climate change. Estuar Coast Shelf S 196:173-181

Wedemeyer GA, McLeay DJ (1981) Methods for determining the tolerance of fishes to environmental stressors. In: Stress and fish, AD Pickering (ed) pp. 247–275 Academic Press, New York

Wendelaar Bonga SE (1997) The stress response in fish. Physiol Rev 77:591-625

Werner EE, Hall DJ (1988) Ontogenetic habitat shifts in bluegill: the foraging rate-predation risk trade-off. Ecology 69:1352-1366

Whitfield AK (1999) Ichthyofaunal assemblages in estuaries: a South African case study. Rev Fish Biol Fisher 9:151-186

Wolanski E, Jones M, Williams WT (1981) Physical properties of the Great Barrier reef Lagoon waters near Townsville. II. Seasonal variations. Mar Freshwater Res 32:321-334

Wong B, Candolin U (2015) Behavioral responses to changing environments. Behav Ecol 26:665-673

Woodland D (1997) Siganidae. Rabbitfishes (spinefoot) pa. 3627–3650. In K.E. Carpenter and V. Niem (eds) FAO Identification Guide for Fishery Purposes. The Western Central Pacific. P. 83

Zarco-Perelló S, Pratchett M, Liao V (2012) Temperature-growth performance curves for a coral reef fish, *Acanthochromis polyacanthus*. Galaxea, Journal of Coral Reef Studies 14:97-103