Regulate or tolerate: Thermal strategy of a coral reef flat resident, the epaulette shark, *Hemiscyllium ocellatum*

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ABSTRACT

Highly variable thermal environments, such as coral reef flats, are challenging for marine ectotherms and are thought to invoke the use of behavioural strategies to avoid extreme temperatures and seek out thermal environments close to their preferred temperatures. Common to coral reef flats, the epaulette shark (*Hemiscyllium ocellatum*) possesses physiological adaptations to hypoxic and hypercapnic conditions, such as those experienced on reef flats, but little is known regarding the thermal strategies used by these sharks. We investigated whether *H. ocellatum* uses behavioural thermoregulation (i.e., movement to occupy thermally favourable microhabitats) or tolerates the broad range of temperatures experienced on the reef flat. Using an automated shuttlebox system, we determined the preferred temperature of *H. ocellatum* under controlled laboratory conditions and then compared this preferred temperature to 6 months of *in situ* environmental and body temperatures of individual *H. ocellatum* across the Heron Island reef flat. The preferred temperature of *H. ocellatum* under controlled conditions was 20.7 ± 1.5°C, but the body temperatures of individual *H. ocellatum* on the Heron Island reef flat mirrored environmental temperatures regardless of season or month. Despite substantial temporal variation in temperature on the Heron Island reef flat (15–34°C during 2017), there was a lack of spatial variation in temperature across the reef flat between sites or microhabitats. This limited spatial variation in temperature creates a low-quality thermal habitat limiting the ability of *H. ocellatum* to behaviourally thermoregulate. Behavioural thermoregulation is assumed in many shark species, but it appears that *H. ocellatum* may utilize other physiological strategies to cope with extreme temperature fluctuations on coral reef flats. While *H. ocellatum* appears to be able to tolerate acute exposure to temperatures well outside of their preferred temperature, it is unclear how this, and other, species will cope as temperatures continue to rise and approach their critical thermal limits. Understanding how species will respond to continued warming and the strategies they may use will be key to predicting future populations and assemblages.

KEYWORDS
climate change, environmental temperature, habitat quality, movement, thermal preference, thermoregulation
The distribution and abundance of species are shaped by the combined effects of abiotic and biotic environmental factors (e.g., Brown, 1984). Tolerance to abiotic factors (e.g., temperature, rainfall, hydrodynamics) typically dictates the range of habitats a species may occupy, while biotic interactions (e.g., competition, predation) influence the abundance of a species within these habitats (Brown, 1984; Tewksbury et al., 2008). Of the abiotic factors, environmental temperature is one of the most important in shaping species distribution patterns (Deutsch et al., 2008; Payne et al., 2016; Sunday et al., 2011), especially for ectotherms, as their rates of biochemical and physiological processes are tightly linked to environmental temperatures (Angilletta, 2009; Pörtner & Peck, 2010). Importantly, it is not only the mean environmental temperature that influences a species’ distribution, but also the variability of environmental temperatures (Tewksbury et al., 2008; Vinagre et al., 2016).

To occupy thermally variable environments ectotherms must tolerate a broad range of temperatures, alter activity and hence energy demands at extreme temperatures (Johansen et al., 2013), and/or use movement to influence their internal body temperature (i.e., behavioural thermoregulation: Casterlin & Reynolds, 1979; Stevenson, 1985; Holland et al., 1992; Crawshaw & Podrabsky, 2011; van de Ven et al., 2019). Of these, behavioural thermoregulation has been shown to be widespread among ectotherms to avoid exposure to suboptimal temperatures (reptiles/fish: Angilletta, 2009; lizard: Carrascal et al., 1992; turtles: Dubois et al., 2009; insect: Nice & Fordyce, 2006). By occupying more favourable thermal conditions, specific physiological processes and/or the performance of the individual as a whole may be optimized (Angilletta, 2009; Fry, 1947). For example, pregnant female Aspic vipers (Vipera aspis) are known to occupy warmer areas than nonpregnant conspecifics, a strategy that is thought to accelerate gestation and decrease development times (Ladyman et al., 2003). Similarly, Gila monsters (Heloderma suspectum) have been shown to select warmer temperatures after feeding to optimize digestion rates (Gienger et al., 2013). Despite the prevalence of behavioural thermoregulation among terrestrial and freshwater ectotherms, studies investigating the potential use of among-habitat movement as a thermoregulation strategy in tropical fishes are limited. Few laboratory studies have shown that tropical reef fish select a preferred temperature under controlled conditions (Habary et al., 2016; Nay et al., 2015, 2018; Reyes et al., 2011; Reynolds & Casterlin, 1981). For example, Reynolds and Casterlin (1981) found eight species of tropical fish had thermal preferences of 20–30°C under controlled laboratory conditions, while Reyes et al. (2011) established a thermal preference of 26.8°C for the bullseye puffer fish, Sphoeroides annulatus. While field studies have investigated the use of among-habitat movements to aid in behavioural thermoregulation strategies (e.g., Aoki et al., 2020), few field studies have demonstrated that neritic tropical fish actively move among habitats to seek out their preferred thermal environment in the field.

Low latitude (i.e., tropical) ecosystems are often characterized as having relatively stable thermal environments compared to those at higher latitudes (Tewksbury et al., 2008). Some tropical environments, however, experience variations in temperature that are comparable to those of temperate ecosystems. Within the coral reef ecosystems, for example, shallow reef flats are known for their extreme thermal variability (Harborne, 2013). Coral reef flats experience dramatic changes in environmental conditions over relatively short temporal scales due to tidal movement and are considered one of the most variable thermal habitats within tropical marine ecosystems (Harborne, 2013; Kinsey & Kinsey, 1967; Nilsson et al., 2007; Potts & Swart, 1984). Although water temperatures on coral reef flats may mirror that of adjacent deeper habitats (e.g., reef slope) during high tide, they can reach temperatures 4°C warmer than these deeper habitats during periods of low tide due to the heating and pooling of shallow water (Chisholm et al., 1996; Harborne, 2013; Potts & Swart, 1984). These elevated temperatures during low tides can lead to daily temperature fluctuations on reef flats in excess of 12°C (Potts & Swart, 1984). While many species move onto and off coral reef flats with the incoming and outgoing tides, respectively (Harborne, 2013; Vivien, 1973), some resident species (e.g., the epaulette shark, Hemiscyllium ocellatum) remain on the reef flat during the entire tidal cycle and are thus exposed to these variable temperatures. The mechanisms that these resident species use to cope with such variable temperatures are largely unknown.

Using a common reef flat resident, the epaulette shark, H. ocellatum, we investigate whether this species uses behavioural thermoregulation or thermal tolerance, similar to other intertidal species (e.g., the mummichog, Fundulus heteroclitus: Fangue et al., 2006; Griffith, 1974), to occupy this thermally variable environment. Given the thermal variability of coral reef flats and the prevalence of behavioural thermoregulation among terrestrial and freshwater ectotherms, we hypothesize that H. ocellatum may also use movement as a behavioural thermoregulation strategy to select more favourable thermal conditions. To test this, we first established the preferred temperatures of H. ocellatum in an automated shuttlebox under controlled laboratory conditions. These preferred temperatures were then compared to the environmental temperatures and internal body temperatures of H. ocellatum individuals on the reef flat of Heron Island, Queensland, Australia (23.4423° S, 151.9148° E) over a 6-month period.

2 | MATERIALS AND METHODS

Heron Island, Queensland, Australia (23.4423° S, 151.9148° E) is a low island within the Capricorn-Bunker group at the southern end of the Great Barrier Reef. It has a large reef flat (~27 km²) that ranges from 0.5 to 2.5 m depth across the tidal cycle (Kline et al., 2015) and has a semidiurnal tidal cycle with a 1.1 m neap tidal range to a 2.3 m spring tidal range (McGowan et al., 2010). Heron Island was selected as it is known to support a large population of H. ocellatum (c. 2200 individuals between 1994 and 1997; Heupel & Bennett, 2007). To investigate the potential for H. ocellatum to behaviourally thermoregulate and/or tolerate the thermally variable environments on the reef flat, we determined the preferred temperatures of individual H. ocellatum under controlled laboratory conditions (T̅pref), and compared the preferred temperatures to the environmental (Tₑ) and internal body temperatures of H. ocellatum (Tᵢ) across the reef flat of Heron Island. The care and
use of experimental animals complied with National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care of Use and Animals for Scientific Purposes, 7th Edition, 2004 and the Queensland Animal Care and Protection Act, 2001 as approved by James Cook University Animal Ethics Committee (#A2316).

2.1 Animal husbandry and preferred temperatures

Epaulette sharks (*H. ocellatum*) were collected by hand during low tide on the reef flat on Heron Island in June 2017 (n = 5) and January 2018 (n = 7). Individual *H. ocellatum* were transported to a 2000 l holding tank at Heron Island Research Station within 60 min of collection and held for a minimum of 3 days prior to being placed inside 10 l plastic bags filled with seawater and oxygen and transported, via car, to the Marine Aquaculture Research Facilities Unit (MARFU) at James Cook University, Townsville, Queensland, Australia. On arrival, sharks were randomly placed into one of five 500 l tanks supplied with 27 ± 1°C (mean ± s.d.) filtered seawater. While *H. ocellatum* were housed in groups of up to five individuals in each holding tank, individual *H. ocellatum* were fed ~4% of their body mass of fresh prawn every second day. *H. ocellatum* were of similar length (57.7 ± 0.7 cm, mean ± s.e.m.) and mass (431.8 ± 24.7 g) regardless of collection season [generalized linear model (GLM): length $z = -0.26, P = 0.79$; mass: $z = -1.58, P = 0.12$] or sex (GLM: length $z = -0.47, P = 0.64$; mass $z = -0.74, P = 0.46$). Prior to shuttlebox trials to determine baseline preferred temperature, individuals were fasted for 48 h to ensure a post-absorptive state (Niimi & Beamish, 1974).

The preferred temperature ($T_{\text{pref}}$) of each *H. ocellatum* was established using an automated shuttlebox system (see Schurmann et al., 1991; Petersen & Steffensen, 2003; Supporting Information Figure S1). In short, the shuttlebox comprised two chambers (120 cm diameter) with a 15 cm opening between the two chambers allowing the shark to travel freely between each chamber. One chamber was designated the “warm” chamber and the other the “cool” chamber. A 2°C differential was maintained between the two chambers (following Schurmann et al., 1991) by organizing the water to flow in a clockwise direction in one chamber and counter-clockwise in the other chamber. To aid in active tracking of each *H. ocellatum* within the shuttlebox system, each individual was fitted with a small elastic harness that held a piece of reflective tape (4 × 2 cm). Preliminary observations revealed that the elastic harness did not affect the shark’s behaviour. A web camera (Microsoft LifeCam HD-3000 Webcam) and four small LED lights were positioned directly above the system, and the camera was connected to a custom program (Labtech Notebook Pro, Laboratories Technology Corp., Andover, MA, USA) to actively track the shark’s position on an $x$-$y$ coordinate system. This program also controlled the rate of heating and cooling of the system by activating and deactivating the appropriate pumps according to the position of the shark. The starting temperature of the warmer chamber was 27°C, the same as the holding tanks, and the cooler chamber was 25°C, 2°C cooler to maintain the temperature differential between chambers. However, once the shuttlebox was activated and when the shark was in the “warm” chamber, the temperature of the entire system was increased by 6°C h$^{-1}$. When the shark was in the “cool” chamber, the temperature of the system was decreased by 6°C h$^{-1}$. The assignment of warm and cool chambers was switched half way through experiments to control for any potential chamber bias.

For each trial, an individual *H. ocellatum* was randomly selected from the holding tanks, placed into the shuttlebox system at 1400 h, and the heating and cooling of the shuttlebox system was activated. Individuals were allowed 19 h to familiarize with the system. Data collection began at 0900 h the following morning and continued for 5 h. The preferred temperature was determined as the temperature at which the individual spent the most time during the 5 h trial (i.e., modal temperature). The lower and upper threshold temperatures were defined as the minimum and maximum temperatures experienced by an individual during the 5 h trial, respectively.

2.2 Environmental and body temperature

The environmental ($T_e$) and body temperatures ($T_b$) of individual *H. ocellatum* were quantified on the coral reef flat at Heron Island. Environmental temperatures were measured for 13 months, starting in January 2017, using 19 HOBO Pendant® Temperature Data Loggers (Onset, Cape Cod, Massachusetts, USA) positioned along two cross-flat transects. Each transect was perpendicular to the shoreline, starting ~50 m from the low water mark and extending to the reef crest (approximately 350–400 m from the shoreline). Temperature loggers were placed both in open (i.e., over sand with no overlying structure) and concealed (i.e., within the coral matrix) microhabitats to investigate potential differences in local thermal environments across the reef flat, areas frequently occupied by *H. ocellatum*. Temperature loggers in open microhabitats were placed at 50 m intervals, while those in concealed microhabitats were placed at 100 m intervals along each transect. Each logger recorded temperature every 30 min and the data were downloaded every 6 months. The first transect, Coral Gardens ($T_{eg}$), was approximately 300 m long and had nine data loggers (i.e., six open microhabitats and three concealed microhabitats), and the second transect, Shark Bay ($T_{eb}$), was approximately 350 m long and had 10 data loggers (seven open microhabitats and three concealed microhabitats). All sharks were collected from the immediate vicinity of the temperature logger transects. To protect and secure the loggers they were placed inside a small PVC tube (length 10 cm, diameter 2.5 cm) with numerous 2–3 cm diameter holes to allow for water movement and cable tied to a concrete block.

To quantify the internal body temperatures ($T_b$) of *H. ocellatum*, 40 individual sharks were collected by hand during low tide on the Heron Island reef flat during June 2017 (n = 30) and January 2018 (n = 10). Captured *H. ocellatum* were maintained in a 2000 l holding tank for a minimum of 24 h prior to being transferred to individual 100 l tanks where they remained for ~10 h prior to tagging and surgical procedures. Individual *H. ocellatum* were anaesthetized in a bath comprising 100 mg l$^{-1}$ tricaine methanesulfonate (MS-222) buffered with 100 mg l$^{-1}$
sodium bicarbonate for 4 min (Smith et al., 2004). Immediately following, a small ventro-lateral incision (~2 cm) was made anterior to the pelvic fins and a temperature data logger (DS1922L iButton Temperature Loggers, Maxim Integrated, San Jose, California, USA) was placed into the abdominal cavity. The incision was sutured closed, and a dart tag (spaghetti tag; Hallprint, Hidden Marsh Valley, South Australia, Australia) was inserted into the dorsal muscle tissue immediately below the anterior dorsal fin to allow for later identification (Heupel, 1997). The surgical procedure took no longer than 6.5 min. Immediately following surgery, sharks were placed into recovery tanks and monitored for a minimum of 24 h prior to being released onto the reef flat at the approximate site of capture. The temperature loggers were set to record body temperatures every 30 min for the following 6 months.

Extensive searches for tagged *H. ocellatum* were conducted on the Heron Island reef flat over a 9-day period in July 2018. During these searches, seven tagged sharks were identified and recaptured by hand (five of the 30 that had been tagged in June 2017 and two of 10 that had been tagged in January 2018). This recapture rate (17.5%), although low, was directly comparable to previously reported recapture rates of *H. ocellatum* at Heron Island (Heupel & Bennett, 2007) and the resulting sample size (*n* = 7) broadly comparable to those used in previous studies investigating the movements of shark and pelagic fish species (*n* = 2: Holland et al., 1992; *n* = 4: Thums et al., 2013; *n* = 4: Payne et al., 2018).

Recaptured sharks were transferred to a holding tank within 2 h of collection where they were held for a minimum of 24 h prior to surgery to remove the temperature loggers. To remove loggers, individual sharks were anaesthetized, as described above, and the implanted iButton temperature loggers were removed. The incision suture was closed and the sharks were placed into recovery tanks for a minimum of 24 h prior to being released on the reef flat.

### 2.3 | Data analyses

All analyses were performed in R (Version 3.4.1, R Core Development Team 2013) using the *lme4* and *mcgv* packages. The most appropriate statistical family and error distribution for each analysis was determined by examining the distribution of the response variable and visually inspecting the residuals for the saturated models. Model assumptions (i.e., homogeneity of variance, collinearity, variance inflation) were assessed using diagnostic plots.

Laboratory-based preferred and threshold temperatures of *H. ocellatum* were compared between collection season and sex.
using GLMs with a gamma distribution. Collection season and sex were fixed effects, and holding tank was included as a random effect.

Environmental temperatures were compared between transects (i.e., Coral Gardens or Shark Bay, fixed factor) and habitat (i.e., open or concealed, fixed factor) using a generalized additive
mixed model (GAMM) with a Gaussian distribution. Day and hour were included with smoother functions to incorporate natural temporal variation (i.e., tidal and diurnal changes). Location (i.e., distance along each transect) was used as the random factor. Due to the nature of the environmental and body temperature data (i.e., non-normal), a Wilcox test was used to compare the environmental and body temperatures of *H. ocellatum*. The analysis was restricted to data from July 2017 to December 2017 as this was the only period for which both environmental and body temperature data were available. All values are reported as means ± S.E.M. unless specifically stated.

3 | RESULTS

Under controlled conditions the preferred temperature of *H. ocellatum* was 20.7 ± 1.5°C (Figure 1a), with upper and lower threshold temperatures of 27.9 ± 0.8°C and 17.7 ± 0.6°C, respectively (Figure 1b). The preferred and threshold temperatures of *H. ocellatum* were consistent between sexes (*T*\textsubscript{pref}: *t* = 1.00, *P* = 0.32; upper threshold: *t* = 0.30, *P* = 0.77; lower threshold: *t* = 11.13, *P* = 0.026) and collection season (*T*\textsubscript{pref}: *t* = −0.09, *P* = 0.93; upper threshold: *t* = −1.58, *P* = 0.11; lower threshold: *t* = 0.62, *P* = 0.53), although the low sample size may have limited the ability to detect differences.

There was a small but significant difference in environmental temperatures between the two transects, with Coral Gardens being, on average, 0.1 ± 0.01°C warmer than Shark Bay (GAMM: *t* = −259, *P* < 0.001). There were, however, no differences between microhabitats (i.e., open vs. concealed; GAMM: *t* = −0.516, *P* = 0.61; Figure 2). Overall, environmental temperatures across the Coral Gardens transect ranged from 15.9 to 33.8°C, with a mean temperature of 24.9°C, while Shark Bay ranged from 15.9 to 33.6°C, with a mean temperature of 24.8°C (Figure 2).

Although limited to a 6-month period and a low sample size, there was no detectable difference between the environmental temperatures and the body temperatures of *H. ocellatum* on the reef flat (*P* = 0.09; Figure 3). The mean deviance of body temperatures from environmental temperature was 0.01 ± 0.34°C (mean ± s.d., range 0.00–2.46°C). Additionally, the frequency of body temperatures largely mirrored that of the environmental temperatures for each of the 6 months (Figure 4).

4 | DISCUSSION

Coral reef flats are thought to be thermally challenging for resident species, but the strategies these species use to cope with such variable temperatures are largely unknown. Despite mean water temperatures on the Heron Island reef flat exceeding the preferred (20.7 ± 1.5°C) and upper behavioural threshold temperature from the shuttlebox trials (27.9 ± 0.8°C) of *H. ocellatum* for 98% and 11% of the year, respectively, the body temperatures of tagged *H. ocellatum* on the reef largely mirrored that of their environment. The confluence of environmental and body temperatures suggests that *H. ocellatum* are not moving off the reef flat to more favourable thermal environments (e.g., reef slope) during high temperatures, but rather that *H. ocellatum* are able to endure a wide range of environmental temperatures (15.9–33.8°C). The lack of evidence for behavioural thermoregulation was perhaps not surprising given the spatial homogeneity of environmental temperature both across the reef flat and between open and concealed microhabitats. *H. ocellatum* are specialized reef flat residents that possess physiological adaptations to tolerate hypoxia and hypercapnia conditions typical of reef flats (see Devaux et al., 2019; Hickey et al., 2012; Mulvey & Renshaw, 2000; Speers-Roesch et al., 2012a,b; Stensløkken et al., 2008) and it appears they are also able to tolerate the wide range of temperatures experienced there.
Coral reef flats, like other intertidal and shallow subtidal habitats, are known to be more thermally variable than adjacent deeper habitats due to the heating and pooling of shallow water during low tides (Harborne, 2013). Although this variation in temperature is primarily temporal, some spatial variation across the reef flat and among microhabitats may be expected due to differences in water depth, proximity to deeper habitats, pooling and shading (Davis et al., 2011; Hearn, 2011). Despite considerable temporal variation in water temperature on the Heron Island reef flat (annual range 15.9–33.8°C), there was limited spatial variation in temperature between sites (0.1 ± 0.01°C) and no detectable difference between microhabitats or across the reef flat. The lack of within-habitat thermal differences may be due to the relatively high water movement and low complexity of habitat on the reef flat, and as such greater spatial variation in temperatures may be evident in less tidally driven and more structurally complex habitats such as deeper forereef or backreef. Given the spatially homogeneity of temperatures across the reef flat, resident *H. ocellatum* did not have the opportunity to access more thermally favourable microhabitats without leaving the reef flat. Indeed, many coral reef fishes are known to migrate from the reef flat to adjacent reef crest and slope habitats during low tides (Gibson, 2003; Harborne, 2013; Unsworth et al., 2007), presumably due to the high temperatures on the reef flat. From the tagged and recaptured individuals in our study, we found no evidence to suggest that *H. ocellatum* were moving from the reef flat to the more favourable thermal environment of the reef slope, despite its proximity. It is possible, however, that nonrecaptured individuals may have emigrated out of the study area, potentially to cooler deeper waters, or died between collection periods. The potential reluctance of the recaptured individuals to move to the reef slope suggests that any physiological benefit of occupying a potentially more favourable thermal environment may be outweighed by the costs associated with other biotic or abiotic factors.

An individual’s habitat selection typically reflects a trade-off between the physiological benefits of selecting a favourable thermal environment and the physiological and/or ecological costs of associated abiotic and biotic factors (Angilletta, 2009). Moreover, moving to a more favourable environment is only beneficial if the movement does not increase the risk of predation, the likelihood of missed opportunities (i.e., allocation of time away from foraging or reproducing) or the energetic costs as a result of moving (Angilletta, 2009). For example, the giant shovelnose ray, *Glaucostegus typus*, and reticulated whipray, *Himantura uarnak*, have been shown to occupy suboptimal thermal conditions to avoid areas of increased predation pressure (Vaudo & Heithaus, 2013). In the present study, *H. ocellatum* may have realized physiological benefits by moving to areas with thermal environments that were closer to their preferred temperature (e.g., the reef slope or outside of the study area). While we found no evidence of such movement in the recaptured individuals, a large proportion of tagged individuals (33 out of 40 individuals) were not recaptured. These nonrecaptured individuals may have died between collection periods, emigrated to other areas or reef, or moved to cooler (e.g., deeper) habitats as a thermoregulation strategy. Heupel and Bennett (2007) suggest that while the epaulette sharks exhibit a high site fidelity, the population at Heron Island may be partially open with some individuals emigrating to other habitats and/or reefs. However, moving to another area may expose individuals to greater risk of predation, as many large, mobile predators are unable to inhabit the shallow coral reef flats, especially during low tide (Harborne, 2013; Rizzari et al., 2014; Vivien, 1973). Additionally, moving to a different habitat such as the reef slope could potentially limit access to prey (e.g., Villén-Pérez et al., 2013) or shelter (e.g., Nielsen & McGaw, 2016), and/or lead to increased competition for resources (e.g., Rusch et al., 2017).

*H. ocellatum* have been shown to have unique physiological strategies to cope with extreme hypoxic conditions (e.g., metabolic depression and neuronal hypometabolism; see Mulvey & Renshaw, 2000; Stensløkken et al., 2008; Devaux et al., 2019), as well as hypercapnia (Heinrich et al., 2014, 2015; Johnson et al., 2016), both of which are evident on shallow reef flats. However, the strategies *H. ocellatum* use to cope with extreme temperatures are unknown. Despite having a relatively low preferred temperature of 20.7 ± 1.5°C under controlled conditions, *H. ocellatum* were able to endure a wide range of temperatures on the Heron Island reef flat (15.9–33.8°C) and experienced temperatures above their upper threshold temperatures for ~11% of 2017. A previous study using a similar shuttlebox system has shown that newly hatched juvenile *H. ocellatum* (total length 14.71 ± 2.23 cm, mass 15.97 ± 2.66 g, mean ± s.d.) move to select their thermal environment during the summer months (thermal preference ~30–32°C), but during the winter months individuals did not move in response to increasing temperatures, even when approaching their critical thermal limits (i.e., 38°C for adult *H. ocellatum*; Gervais et al., 2018). Although we did not detect an effect of collection period on the preferred temperature of adult *H. ocellatum*, the cooler preferred temperature in the present study may be related to the significantly larger body size (and age) of individuals, the longer holding time (up to 8 months) and/or the fasting prior to experimentation. The ability to occupy such a thermally variable environment indicates that *H. ocellatum* may possess other physiological mechanisms (e.g., heat-shock proteins; Roberts et al., 2010, Tomanek & Somero, 1999; Tomanek, 2010) that allow them to tolerate variable temperatures.

Occupying thermally variable environments, such as coral reef flats, is assumed to be challenging for ectotherms. While behavioural thermoregulation appears common among larger mobile marine fishes and sharks who move among habitats to occupy thermally favourable conditions (e.g., bigeye tuna: Holland et al., 1992; whale shark: Thums et al., 2013; tiger shark: Payne et al., 2018; juvenile yellowfin tuna: Aoki et al., 2020), this might not be the case for most small-bodied site-attached species, such as *H. ocellatum* (for an exception see Gibson et al., 2015). Rather, smaller, site-attached species may rely on physiological adaptations to endure such extreme thermal environments. Our records show that *H. ocellatum* were exposed to temperatures upwards of 35°C on the Heron Island reef flat, a temperature that is approaching the critical thermal limit for this species (c. 38°C; Gervais et al., 2018). Irrespective of the adaptations to acute fluctuations in temperature, predicted increases in the frequency and...
duration of exposure to elevated temperatures under ongoing climate change (Collins et al., 2013) are likely to be challenging for reef flat residents. Understanding how *H. ocellatum* and other reef flat residents cope with current and future temperatures will be key to predicting future populations and distributions.

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**CONTRIBUTIONS**

T.J.N., C.R.G, J.L.J., J.L.R. and A.S.H conceived and designed the experiments. T.J.N., C.R.G. and R.J.L. performed the experiments and analysed the data, and T.J.N., C.R.G. and A.S.H wrote the manuscript. All authors provided significant technical support and editorial guidance.

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