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Title: Adapt, move, or die – how will tropical coral reef fishes cope with ocean warming?

Running head: Thermal preference of coral reef fishes

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Key words: acclimation, aerobic scope, behavioural thermoregulation, critical thermal limits, global warming, temperature preference

Abstract

Previous studies hailed thermal tolerance and the capacity for organisms to acclimate and adapt as the primary pathways for species survival under climate change. Here we challenge this theory. Over the past decade more than 365 tropical stenothermal fish species have been documented moving pole-ward, away from ocean warming hotspots where temperatures 2-3 °C above long-term annual means can compromise critical physiological processes. We examined the capacity of a model species - a thermally-sensitive coral reef fish, Chromis viridis (Pomacentridae) – to use preference behaviour to regulate its body temperature. Movement could potentially circumvent the physiological stress response associated with elevated temperatures and may be a strategy relied upon before genetic adaptation can be effectuated. Individuals were maintained at one of six temperatures (23, 25, 27, 29, 31 and 33 °C) for at least six weeks. We compared the relative importance of acclimation temperature to changes in upper critical thermal limits, aerobic metabolic scope, and thermal preference. While acclimation temperature positively affected the upper critical thermal limit, neither aerobic metabolic scope nor thermal preference exhibited such plasticity. Importantly, when given the choice to stay in a habitat reflecting their acclimation temperatures or relocate, fish acclimated to end-of-century predicted temperatures (i.e., 31 or 33 °C) preferentially sought out cooler temperatures, those equivalent to long-term summer averages in their natural habitats (~29 °C). This was also the temperature providing the greatest aerobic metabolic scope and body condition across all treatments. Consequently, acclimation can confer plasticity in some performance traits, but may be an unreliable indicator of the ultimate survival and distribution of mobile stenothermal species under global warming. Conversely, thermal preference can arise long before, and remain long after, the harmful effects of elevated ocean temperatures take hold and may be the primary driver of the escalating pole-ward migration of species.

Introduction

Over the evolutionary history of vertebrates, adaptation to thermal changes has occurred at a rate of approximately 1 °C per million years **(**Quintero & Weins, 2013). As current ocean temperatures and rates of warming exceed those over the past 420,000 years (Hoegh-Guldberg *et al*., 2007; Collins *et al.*, 2013), the fundamental biogeographical principle of "adapt, move, or die" has perhaps never been more relevant. Global climate change is not only predicted to increase average sea surface temperatures (SSTs) by 2.0-4.8 °C by the end of the 21st century but also increase the intensity and frequency of transient thermal fluctuations (Ficke *et al*., 2007; Ganachaud *et al*., 2011; Wernberg *et al*., 2012; Collins *et al.*, 2013; IPCC 2013). Organisms living at extreme latitudes (i.e., near the equator or poles) are expected to be particularly sensitive to predicted thermal changes because these species are thought to have evolved within narrow and stable temperature ranges (Huey & Kingsolver, 1993; Tewksbury *et al*., 2008; Gardiner *et al*., 2010; Lough, 2012). Consequently, these species may also be less capable of acclimating and/or adapting to contemporary temperature changes, particularly over the short time scales predicted with global warming (Pörtner, 2002; Stillman, 2003; Somero, 2010; Neuheimer *et al*., 2011; Nguyen *et al*., 2011; Rummer *et al*., 2014), highlighting a serious threat to long-term fitness and survival of countless species unless relocation to more favourable thermal habitats is possible.

Reversible thermal acclimation, which occurs over days to months, usually in response to daily or seasonal changes, can allow organisms to cope with changes in temperatures by enhancing important performance traits (Kinne, 1962; Angilletta *et al*., 2006; Sandblom *et al*., 2014). Most species examined appear to adopt this strategy. For example, the Columbia River redband trout (*Oncorhynchus mykiss gairdneri*) significantly reduces its heat shock response to repeated thermal stress following six weeks of acclimation (Narum *et al.,* 2013). However, while acclimation is recognized as a primary driving force for adaptive phenotypic changes

(Gienapp *et al*., 2008; Teplitsky *et al*., 2008; Hoffmann & Sgró, 2011; Culumber & Monks, 2014), acclimation may come at a cost, i.e. may involve detrimental energetic trade-offs (Angilletta, 2009; Donelson *et al*., 2011, 2014) or not happen fast enough to keep pace with the rate of environmental changes (Angilletta, 2009; Chown *et al*., 2010; Quintero & Weins 2013; Sandblom *et al*., 2014).

Capacity for acclimation may be extremely limited for stenothermal tropical coral reef fishes; studies have already shown that temperatures just 2-3 °C above annual summer maxima can compromise a wide range of life-history traits including swimming, growth, activity, and reproduction (Munday *et al*., 2008; Johansen & Jones, 2011; Donelson *et al*., 2012b; Zarco-Perello *et al*., 2012; Johansen *et al*., 2013, 2015; Rummer *et al*., 2014). These important traits are supported by the animal's aerobic metabolic scope (AMS), which is essentially the capacity to direct energy toward critical tasks beyond that required for basic maintenance. In principle, AMS is defined as the difference in oxygen consumption rates ($\dot{M}O_2$) between resting and maximal performance (Fry & Hart, 1948; Pörtner & Farrell, 2008) and is thought to follow a right skewed, bell-shaped curve with temperature (Pörtner & Knust, 2007). A stenothermal species will typically have the greatest AMS at a particular temperature (T_{opt}), beyond which AMS declines, perhaps due to cardiorespiratory limitations that reduce O_2 supply to the tissues (Pörtner & Knust, 2007). Transient heating events – for example, those associated with El Niño – can elevate temperatures beyond the thermal tolerance of a species and consequently reduce AMS to near zero, causing imminent death as fish lose the ability to maintain bodily functions (Beitinger *et al*., 2000; Ospína & Mora, 2004; Pörtner & Knust, 2007).

While an overall loss of AMS is detrimental to a species (Pörtner, 2001, 2002; Pörtner & Knust, 2007), some physiological processes are likely to be more thermally sensitive than others and may consequently be impacted at temperatures closer to optimum than those tolerated from a whole-animal perspective (see Steinhausen *et al*., 2008; Casselman *et al*., 2012; Iftikar & Hickey, 2013; Iftikar *et al*., 2014). This phenomenon is sometimes interpreted as the “multiple performances – multiple optima” theorem (see, e.g., Clark *et al*., 2013). For instance, the common coral reef damselfish (*Acanthochromis polyacanthus*) exhibits a reduction in aerobic scope when reared at 1.5 °C above their summer average temperatures, but still maintains reproductive output at this temperature, suggesting a mismatch between the thermal optimum for metabolic enzymes and reproductive hormones (Donelson *et al*., 2014). Thus, many populations may be sensitive to relatively small increases in SSTs at some aspect of their performance, which can jeopardize biological fitness and population sustainability (Pörtner & Farrell, 2008; Pörtner & Peck, 2010).

Numerous studies have examined the short- and long-term physiological impacts of elevated SSTs on tropical coral reef fishes, but many species – if not most – are also capable of behavioural thermoregulation (Casterlin & Reynolds, 1980; Reynolds & Casterlin, 1981; Angilletta *et al*., 2006). An organism can behaviourally thermoregulate by moving out of a challenging thermal habitat (e.g., across a thermocline) and into a different thermal habitat where temperatures are more optimal (T_{opt}) (Reynolds & Casterlin 1979; Johnson & Kelsch, 1998; Khan & Herbert, 2012). Given that AMS and the associated capacity for movement, swimming, etc. are maximized at a specific range of body temperatures, individuals that seek out habitats where this body temperature can be maintained will, in theory, maximize fitness (Kelsch & Neill, 1990; Johnson & Kelsch, 1998; Angilletta *et al*., 2002).

Physiological thermal sensitivity and thermoregulatory behaviour appear to be co-adapted (Huey & Bennett, 1987; Angilletta *et al*., 2002, 2006) as the thermal history that defines a species’ T_{opt} often determines its preferred temperature range (Kelsch & Neil, 1990; Johnson & Kelsch, 1998). Therefore, in nature, most species are likely to preferentially pursue temperatures that coincide with their T_{opt} during a given life stage (Brett, 1971;

Beitinger & Fitzpatrick, 1979; Jobling, 1981; Pörtner & Knust, 2007; Pörtner & Farrell, 2008; Payne *et al.*, 2016). Critically, for behavioural thermoregulation to help mitigate the effects of rapid climate change, evolutionary changes in T_{opt} should also provide a strong selective pressure for changes in T_{pref} (Kelsch & Neill, 1990; Angilletta *et al*., 2002). This inherent relationship between T_{opt} and T_{pref} has not previously been examined in thermally-sensitive species such as coral reef fishes, and it remains uncertain whether phenotypic shifts in temperature sensitivity of aerobic performance (e.g., see Donelson *et al*., 2011, 2012a) also lead to changes in T_{pref}.

It is critically important to understand the relative roles of acclimation and behavioural thermoregulation in order to predict how warming ocean temperatures will ultimately impact fitness and distribution of individual species. As oceans warm, cooler conditions more closely aligned with a species T_{opt} may be found in deeper aquatic habitats or at higher latitudes, suggesting that species distribution ranges will shift accordingly (Perry *et al*., 2005; Booth *et al*., 2007; Figueira & Booth, 2010; Wu *et al*., 2012; Jones *et al.*, 2014). Indeed, more than 365 tropical reef fish species have already been identified to be expanding their latitudinal ranges pole-ward at a rate of up to 26 km per decade (Figueira & Booth, 2010; Feary *et al*., 2013; Nakamura *et al*., 2013). However, for the numerous other coral reef species that are either partly or completely reliant on coral reefs for survival, higher latitudes may not provide suitable habitats. Such species will have to follow the distribution changes of coral and other benthic flora and fauna that are less mobile than fish species and expected to relocate at a slower pace, if at all. The survival of such habitat-dependent species may consequently be predicted by their capacity to acclimate their T_{opt} and T_{pref} to projected temperatures.

Focussing on a model species – a thermally-sensitive coral reef fish, *Chromis viridis* (Pomacentridae) – we examined the coordinated adjustments of T_{opt} and T_{pref} to elevated temperatures expected within generational timescales and the propensity of individuals to remain or evade elevated temperatures. We tested three hypotheses: (i) the preferred temperature (T_{pref}) of a tropical stenothermal coral reef fish will coincide with their optimal temperature (T_{opt}) for aerobic performance (specifically aerobic scope); (ii) prolonged thermal acclimation will improve some, but not all aspects of metabolic performance at elevated temperatures; and (iii) the thermal preference of this stenothermal coral reef fish will be fixed and a stronger behavioural driver than phenotypic acclimation, causing individuals to move toward established optimal temperatures. These hypotheses are thus focusing on short term/localized movements, yet the results could provide explanations for whole animal responses, including range shifts.

Materials and methods

Study species, collection, and holding conditions

The model species – a thermally sensitive coral reef fish, the blue-green damselfish, *Chromis viridis* – is a representative of one of the most species-rich families (Pomacentridae, >360 spp.) of coral reef fishes and are known to occupy an array of habitats in both tropical and subtropical environments around the world. This species is highly abundant and widely distributed on coral reefs (~27°N to 27°S) at depths of 12m or shallower (Randall *et al*., 1997). This species is highly site-attached, and at all post-larval life stages, is closely associated with corals (typically *Acropora* spp.) that provide shelter from predators and a nocturnal retreat (Fishelson *et al*., 1974).

In January 2014, a total of 72 individuals exhibiting adult coloration and of equal body mass (4.63 ± 1.23g; mean ± SD), were collected using monofilament barrier nets from reef crest sites around Lizard Island (14° 40’ 08"S, 145° 27’ 34"E) in the northern part of the Great Barrier Reef, Australia under Marine Parks Permit #G10/33239.1. The annual sea surface temperatures (SST) of this area range from 23.5 to 30.0 °C (data averaged monthly since 1982, summarized in Rummer *et al*., 2014 and Johansen *et al.*, 2015).

All individuals were transported to the Marine Aquaculture Research Facilities Unit (MARFU) at James Cook University (JCU) in Townsville, Queensland, Australia and randomly distributed between six 100 L cylindrical tanks (65 x 40 cm, height x diameter) with 12 individuals per tank. Tanks were maintained under a 12:12 light:dark photoperiod and continuously supplied with recirculated, filtered, aerated, and UV-sterilized sea water (34 ppt, 28 °C). Fish were fed to satiation twice daily with commercial pellets and hatched *Artemia* spp. (NRD 500-800 µM, INVE Aquaculture®, Salt Lake City, USA) and provided with multi-sized shelters in which to hide. Tanks were cleaned on a daily basis. Then, at least one week prior to experimentation, each fish was tagged with visible elastomer implanted into the epaxial muscle or caudal region (Northwest Marine Technology®, Inc., Shaw Island, USA), which made it possible to identify individuals for repeated measures experimentation. Throughout the duration of the project, fish were maintained under James Cook University Animal Ethics Committee regulations (permit: #A2089, approved for this study)

Temperature treatment

One week following tagging, all tanks were randomly subdivided across six temperature treatments representing the annual temperature range that this population of *C. viridis* experiences near Lizard Island (23, 25, 27 or 29 °C) in addition to two higher temperatures (31 or 33 °C) corresponding to the 2.1-4.0 °C projected increase in SST by 2100 (Collins *et al*., 2013). Temperatures were reduced or increased by 0.5 °C day^{-1} until target temperatures were reached. Water for the 29, 31, and 33 °C treatments was heated using 300 W submersible heaters (JEBO®, Guangdong, China) that were controlled by automated temperature controllers (N323, NOVUS Automation®, Porto Alegre, Brazil). Water for the

23, 25, and 27 °C treatments was cooled using external water chillers (HC-130A Hailea®, Guangdong, China). Temperatures were manually checked three times daily and always maintained within a range of ± 0.2 °C.

Fish were maintained at each target temperature for a minimum of six weeks prior to experimentation so that acclimation at the level of metabolism, if any, would be presumed complete by this time (Nilsson *et al*., 2010). To determine growth trajectories, all fish were weighed at the initial point of tagging, following each experiment, and at the conclusion of the 27-week experimental period. Prior to all experimental trials, each individual was starved for 24h to ensure a post-absorptive state that maximized energy available for performance (Niimi & Beamish, 1974; J.L. Rummer, unpublished data).

Metabolic rates and the scope for aerobic metabolism

Intermittent flow respirometry was used to estimate maximal metabolic rates (MMR), standard metabolic rates (SMR) and aerobic metabolic scopes (AMR) of eight randomly selected individuals from each temperature treatment. All protocols followed Steffensen (1989) and Rummer *et al.* (2016). The MMR was estimated from the maximal oxygen consumption rate measured immediately after a fish was exercised by chasing. The SMR was estimated from the oxygen consumption rate of a fish at rest, and AMS was calculated as the difference between MMR and SMR (see also Clark *et al*., 2012, 2013; Roche *et al.*, 2013; Rummer *et al.*, 2016 for details on these protocols).

Metabolic rates were estimated using well established methodologies and techniques that all aim at ensuring low levels of systemic interference. In short, each fish was first chased continuously by hand for 3 min in a 100L circular (65cm x 40cm: height x diameter) aquarium containing well-aerated and temperature-controlled seawater maintained at the fish’s treatment temperature. During this time, the experimenter would only touch the tail of the fish if it slowed down or stopped swimming. Individuals were considered exhausted when they became unresponsive to chasing, which always occurred before the end of the 3-min chase period. The fish was then scooped into a rubber mesh net and maintained out of the water for 1 min to necessitate anaerobic metabolism (Clark *et al.*, 2013; Roche *et al.*, 2013; Rummer *et al.*, 2016). Following air exposure, individual fish were immediately placed into one of eight custom-built cylindrical acrylic respirometry chambers (each ~360 ml in total volume, 21.6 x 4.6 cm; length x diameter) submerged in a temperature-controlled bath (5000 W heaters, Control Distributions®, Carlton, Australia or HC-1000A chillers, Hailea®, Guangdong, China, via sump). Each respirometry chamber was sealed within 5 seconds where after, reductions in O_2 concentration in the respirometry chambers were measured for the following 7-min. The maximal oxygen consumption rate (i.e., MMR) was calculated from the steepest 1-min slope during this 7-min interval. Then, the respirometry chamber was flushed with fully oxygenated seawater for 7-min before a new 7-min measuring cycle was initiated. The fish was maintained in the respirometry chamber for 22-24 h until O_2 consumption rates had stabilized and no longer decreased (Rummer *et al*., 2016). During this time, each repeat 14-min flush-measuring cycle was controlled by a digital relay timer (MFRT-1 Multi Function Recycling Timer, Xiamen SUPERPRO Technology Co., Ltd., Xiamen, Fujian, China) connected to submersible flush pumps (Eheim®, Germany, 200L h^{-1}). The measurement period was short enough to ensure that O_2 within the respirometry chamber always remained above 80% air saturation, which is important to avoid metabolic changes associated with hypoxia (Hughes, 1973, Tetens & Lykkeboe, 1985, Boutilier *et al.*, 1988). The flush period was long enough to ensure oxygen levels returned to 100% air saturation. Each of the eight respirometry chambers was also connected to its own in-line pump (Aquapro® AP200LV, 200 L h^{-1}) to continuously recirculate water within the chamber at all times regardless of flushing cycle, thus ensuring complete mixing and homogenous water

PO_2. All water used for experiments was first sterilized using a 36 W UV filter (Blagdon Pro UVC 16200®, China) to minimize bacterial growth within the respirometry chambers, but in addition, all chambers, tubing, and pumps were flushed with chlorinated (10% bleach) freshwater and sundried for at least 8h every 24-48h. Bacterial respiration within the chambers (i.e., the drop in O_2 concentration within an empty chamber) was quantified in each chamber prior to and after each trial but, because of the regular cleaning, never exceeded 10% of $\dot{M}O_{2\ (rest)}$ of the fish. During all trials, each respirometry chamber was partly covered using a cylinder of black plastic with a 2 x 12 cm window. This setup allowed light to enter the chamber but prevented fish from being disturbed via external visual stimuli.

The O_2 concentrations (temperature and barometric pressure compensated) in each respirometry chamber were measured using a Firesting Optical Oxygen Meter (two four-channel units, Pyro Science e. K.®, Aachen, Germany) and associated manufacturer's software (via PC computer) at a rate of 0.5 Hz and saved as text files. After each trial, raw Firesting text files were imported into Lab Chart version 6.1.3 (AD Instruments®, Colorado Springs, CO, USA) for analysis. The $\dot{M}O_2$ in mg O_2 kg^{-1} h^{-1} at each time point over the 22-24h trial was calculated based on the volume of the respirometry chamber, volume of fish, and mass of fish following equations of Bushnell *et al*., (1994) and Schurmann and Steffensen (1997). Specifically, maximal metabolic rate estimated from the first measuring period as describe above. The remaining ~94 measuring periods were used to estimate SMR based on the "mean of the leftmost normal distribution" method (MLND) of Chabot *et al.*, (2016), which is understood to be a rigorous and accurate way to estimate SMR (see Svendsen *et al.*, 2016). In short, this method detects the bimodal normal distribution of oxygen consumption rates over the 22-24h recovery period and assigns the peak of the leftmost distribution curve as SMR.

Only slopes with $R^2 \geq 0.90$ were used in order to reduce the impact of systemic variations in O_2 measurements (Svendsen *et al.*, 2016). The proportional background O_2 consumption rate (measured as O_2 depletion in the empty respirometry chambers before and after each trial, assumed linear) was subtracted from each $\dot{M}O_2$ measurement. Temperature quotients (Q_{10}) were calculated according to Clarke and Johnston (1999) to evaluate the level of temperature dependence of SMR at the higher end of the thermal range.

Critical thermal maxima

Upper critical thermal limits (i.e., CT_{Max}) were determined for 40 randomly selected fish – specifically, 8 fish from the 23 °C treatment, 8 from 25 °C, 7 from 27 °C, 6 from 29 °C, 7 from 31 °C, and 4 from 33 °C – using critical thermal methodology (CTM; Beitinger *et al.*, 2000). The low sample size at 33 °C was due to low survival rates at this temperature (see discussion). Experiments were performed using four 3-L containers (each holding one individual) suspended within a 100-L water bath. Containers were pierced on all sides, allowing seawater to flow through readily, and each container also contained an air stone to ensure sufficient aeration. The water bath was provided with a continuous supply of clean, aerated, and UV-filtered seawater, which was heated (5000 W, Control Distributions®, Australia) or chilled (HC-1000A, Hailea®, China) to the fish's respective treatment temperature prior to commencing experimentation. To ensure sufficient mixing, two pumps (WH-500, Weipro®, Guangdong, China) were placed on the bottom of the water bath. Pilot trials confirmed that temperatures remained the same between all four 3-L containers throughout experimentation. After being placed in their respective containers, the four fish were left to habituate to the containers for approximately 15 min., after which time, water temperature was increased by 0.28 ±0.03 °C min^{-1} until fish lost equilibrium. Loss of equilibrium (LOE) has been previously defined as when a fish loses its inability to right itself (see Beitinger *et al.,* 2000). Upon LOE, each fish was quickly removed from its respective container using a mesh net and placed into a separate aquarium to recover at its original treatment temperature. The temperature at which each fish reached LOE was recorded in each container using a digital thermometer (Type T C26 (temperature resolution: 0.1 °C), Comark®, Norfolk, United Kingdom).

Temperature preference

The preferred temperature (T_{pref}) of 48 individual fish from each temperature treatment (i.e., 23, 25, 27, 29, 31 and 33 °C) was determined using a custom-designed two-chamber shuttlebox system (developed by J.F. Steffensen and described in detail in Nay *et al.*, 2015, but see also Schurmann *et al.*, 1991; Schurmann & Steffensen, 1994; Petersen & Steffensen, 2003). This setup allows a fish to use movement to control the temperature of its water and therefore its body temperature (i.e., preferred temperature; T_{pref}). In brief, the system consisted of a "hot" chamber and a "cold" chamber, each made from white PVC walls and a transparent Plexiglas bottom (ø35 cm). A 10 cm x 5 cm opening in the wall joined the two cylindrical chambers (i.e., like a figure-eight), and the fish was able to swim freely between the two chambers. The temperature differential between the two chambers was always maintained at 1.5-2 °C (using two submersible pumps, WH-500, Weipro®, Yongcheng Aquarium, China), which ensured that the fish could detect a temperature difference when selecting between the two chambers (J.F. Steffensen, personal observation). When the fish entered the "hot" chamber, the temperature of both chambers would increase at a rate of 6 °C h^{-1} until the fish moved to the "cold" chamber, which would be 1.5-2 °C cooler. While the fish was in the "cold" chamber, the temperature of both chambers would cool at a rate of 6 °C h^{-1} until the fish moved back to the "hot" chamber, which was 1.5-2 °C warmer. By swimming back and forth between the two chambers, the fish could effectively control the temperature of its environment and thus its own body temperature.

The temperatures for each cylindrical chamber were maintained by pumping in water (480 L h^{-1}, pump HX-6510, Guangdong, China) from corresponding external buffer tanks (ø10 cm), which then passively returned from each chamber to its corresponding buffer tank. Inlets and outlets in each chamber were constructed to ensure water flowed in a clockwise direction in one chamber and a counter-clockwise direction in the other chamber, which prevented unwanted mixing of water between the “hot” and the “cold” chambers in the connecting wall opening. The temperatures of the buffer tanks were controlled by pumping water at a rate of 420 L h^{-1} (WH-500, Weipro®, Yongcheng Aquarium, Guangdong, China) through stainless steel spirals from the buffer tanks into cooling and heating reservoirs, and back. The reservoirs were either heated to 45 °C (5000W heater, Control Distributions®, Carlton, Australia) or externally chilled to 5 °C (HC-130A, HC-1000A, Hailea®, Guangdong, China and 1/3 hp Aqua One Arctic, Aqua One®, Southampton, UK). The water level in all compartments of the system was ~ 20 cm (total system volume of ~42 L), and the entire setup was shielded with black plastic sheeting to protect fish from external visual stimuli while still maintaining 12:12 light:dark photoperiod.

During each trial, temperatures within the “hot” and the “cold” chambers were continuously recorded (5 Hz) using inline thermocouple sensors mounted along the inside chamber walls. The temperature sensors were connected to temperature readers (PR-5714, PR Electronics®, Rønde, Denmark) and a computerized software system. Above the shuttlebox setup, a mirror angled downward at 45° allowed video monitoring of fish movements via a video camera (SONY® HDR-XR100E) mounted on the opposite wall (4.75 m distance). Infrared lights mounted below the transparent Plexiglas bottoms were used to illuminate the chambers from below to create a detectable contrast between the fish and its surroundings. A

PC video frame-grabber (USB 2.0 DVD maker®) transmitted the video signal from the digital video camera to a laptop. Then, by using position analyzer software (LoliTrack, Loligo Systems®, Tjele, Denmark), the position of the fish was continuously tracked, thus allowing the custom-designed software (Labtech Notebook) to automatically heat or cool the two chambers based on the real-time movement of the fish (for further details, see Schurmann *et al*., 1991; Schurmann & Steffensen, 1994). During the night, a small lamp provided a level of light similar to full moon on the reef, which ensured that fish could continue to navigate between the two chambers.

The length of time necessary for fish to learn the shuttlebox system and select T_{pref} was determined via a series of 48 h pilot trials using fish from two of the temperature treatment groups (25 and 31°C). As a result, a 24 h experimental period was chosen because T_{pref} stabilized within 24 h in all pilot trials and did not change with longer exposure time (paired t-test; $P_{25°C} = 0.253$, $P_{31°C} = 0.742$).

Following the 24 h fasting period, individual fish were placed into the shuttlebox system set to their respective treatment temperature for 1.5 h to habituate to the aquaria without experiencing any change in water temperature. Movement-controlled heating/cooling patterns commenced at 1830 each evening and continued until 1630 the following day. To preclude the potential effects of specific dynamic action on T_{pref} (Wallman & Bennett, 2006) no food was provided 24h prior to or during experimentation. For the night time periods (1830 until 0630 the following day), reservoir temperatures were set so that the shuttlebox would not continue to heat or cool the fish past lethal limits (A. Habary, personal observation). This meant that if the fish did not move appropriately between the two chambers at night, the shuttlebox system would not inadvertently kill the fish while the computer waited for the fish to move. During daylight hours (from 0630 – 1630), however, when the observer was able to regularly monitor the fish, reservoir temperatures could exceed the fish's lethal thermal limits. Following each trial, all tanks were rinsed with clean seawater to eliminate olfactory cues from the previous fish. After half of all trials were completed for each treatment temperature, the "hot" and "cold" chambers were switched to eliminate spatial bias.

The T_{pref} data were analyzed by averaging the selected temperature range for every 10 minutes during the last 5 h of each trial for each fish. Means were then compared between the fish from each treatment temperature (similar to the methodology described by Killen 2014). If the tracking software was unable to detect the fish movement for more than one minute (e.g., if the fish was positioned too close to the chamber wall), then the missing time period was excluded from analysis in addition to the same amount of data following the missing time period (Nay *et al.*, 2015). During the time period when the tracking software could not detect the fish, the system would continue to increase or decrease the temperature. Removing these missing data as well as the following time period allowed for the system to stabilize back to the original point where the fish was 'lost' and to compensate for any change in temperature caused by the system's inability to track the fish.

Data analyses

The SMR, MMR, AMS and the effect of body mass were all analyzed using general linear models (GLM) with SMR, MMR, and AMS as dependent variables, treatment as a categorical predictor, and body mass as a continuous predictor. The CT_{Max} data were analyzed using a one-way ANOVA followed by the Tukey HSD test, while mean body mass values within and between temperature treatments were compared using a one-way ANOVA and, when necessary, Holm-Sidak *post hoc* tests. The final T_{pref} values between groups were analyzed using a nonparametric Kruskal Wallis test, and differences between treatments were compared using the Tukey HSD test. Growth data (mass loss/gain over the 27-week project) was analyzed using a one-way ANOVA and, when necessary, Holm-Sidak *post hoc* tests. Linearity, normality and homoscedasticity were verified for all data sets using residual-fit plots. When necessary, variables (SMR, MMR and AMS) were $\log_{10}$ transformed for use in models. The level of significance was $\alpha = 0.05$ for all tests, and all statistical analyses were performed using Statistica v. 12 (Statsoft Inc., Dell®, Tulsa, Okla., USA).

Results

Metabolic rates and aerobic metabolic scope

Treatment temperature did not significantly influence aerobic metabolic scope (AMS) (Fig. 1b; GLM, $F_{5,47} = 2.25$, $P = 0.06$) despite a 31.2% and 30.2% reduction in AMS between fish maintained at 33 °C versus fish maintained at 27 or 29 °C, respectively.

By contrast, treatment temperature did significantly influence estimates of both standard metabolic rate (SMR) (GLM, $F_{5,47} = 5.89$, $P < 0.01$) and maximum metabolic rate (MMR) (Fig. 1a; GLM, $F_{5,47} = 2.85$, $P = 0.02$). Here, SMR increased by ~86% from 23 °C to 31 °C before decreasing again at 33 °C (Fig. 1a), and MMR increased by up to 36.4% between 23 and 29 °C. Yet temperature only had an overall effect on MMR as there were no statistically significant differences between individual treatments (Fig. 1a). The temperature quotient (Q_{10}) calculated for SMR between 29 and 31 °C was 2.06 but fell to 0.97 for SMR estimates between 29 and 33 °C and 0.46 between 31 and 33 °C.

Critical thermal maxima

There was a significant positive effect of treatment temperature on CT_{Max} ($F = 42.55$, $P < 0.01$). Specifically, CT_{Max} increased by approximately 0.5 °C for every 1 °C increase in treatment temperature (Fig. 2a, regression equation: $CT_{Max} = 0.45x + 25.2$, x = °C, $R^2 = 0.97$) with average CT_{Max} values ranging from 35.4 ± 0.2 °C (mean ± SEM) for fish maintained at 23 °C to 39.2 ± 0.2 °C (mean ± SEM) for fish maintained at 31 °C (Fig. 2a).

Importantly, fish from the 33 °C treatment reached a mean CT_{Max} of 40.5 °C, but were unable to recover from this exposure and died within 24 h following experimentation. One of the criteria, i.e., the ability of an animal to escape from conditions that will lead to its death, for CTM (see discussion in Beitinger *et al*., 2000) was therefore not met, and consequently these data were excluded from analyses however still depicted in Fig. 2a for reference.

Temperature preference

Treatment temperature had a significant effect on the average T_{pref} of *C. viridis* when compared across all treatments (mean T_{pref} = 28.9 °C, Kruskal-Wallis, H = 15.7, $P < 0.01$; Fig. 2b). However, only the T_{pref} of the 23 °C and 33 °C treatment groups differed from one another (T_{pref} = 26.1 °C ± 0.96 and 30.4 °C ± 0.37, means ± SEM, respectively, $P = 0.04$; Fig. 2b), and neither the T_{pref} of fish at 23 °C nor the T_{pref} of fish at 33 °C were statistically distinct from the T_{pref} of fish from 25, 27, 29, or 31 °C ($P = 0.31$). The overall mean T_{pref} for all treatment groups was 28.9 °C, which coincided with the optimum temperature (T_{opt}) for AMS (27-29 °C) (Figs. 1b and 2b).

Growth

Temperature treatment had a significant overall effect on body mass (Fig. 3, $P<0.01$). Specifically, fish that were maintained at temperatures they would normally experience in the wild (i.e., 23, 25, 27, or 29 °C) had increased their body mass by 15.4, 16.7, 19.2 and 15.7%, respectively after 27 weeks ($P = 0.04$). By comparison, fish maintained at 31 °C showed no change in body mass ($P < 0.05$), while fish maintained at 33 °C lost, on average, 30% of their body mass ($P = 0.02$).

Discussion

In a time of rapid global warming, the capacity for acclimation has been hailed as the primary pathway for species survival. Here, we highlight another mechanism by demonstrating that a model species – a thermally-sensitive coral reef fish, *Chromis viridis* (Pomacentridae) – appears to lack the ability to acclimate at the level of metabolic rate, but instead maintains a behavioural preference and actively seeks out habitat temperatures that maximize metabolic performance and growth, even after prolonged exposure to water temperatures outside of this range. This is the first time thermal preference for lower ambient temperatures has been shown to persist after prolonged acclimation to elevated temperatures, thus providing one explanation for the rapid pole-ward expansion of thermally sensitive species worldwide (Feary *et al.* 2013).

Modifying behaviour to select preferred temperatures can be one of the initial responses an organism has to elevated temperatures (Olla *et al*., 1978; Sloman & McNeil, 2012). Multiple factors can potentially modify temperature preferences (e.g., sex and individual variation, see Wallman & Bennett, 2006; Podrabsky *et al*., 2008; Biro *et al*., 2010; Killen, 2014), and here we show that a narrow thermal range for optimal metabolic performance confers an equally narrow range of preferred temperature (see also Angilletta *et al*., 2006). Despite a minimum of six weeks of acclimation to one of six temperatures spanning the total yearly temperature range experienced by these individuals in the wild as well as global warming projections, most individuals maintained a preference for the exact same temperature range found in their natural habitat during the summer months. This is likely the result of strong evolutionary selection for and adaptation to a narrow and stable thermal range (Johnson & Kelsch, 1998; Angilletta *et al*., 2002, 2006). The thermal range for which species are adapted indicates the temperature range that maximizes fitness and thus promotes optimal growth, reproduction, swimming, etc. (Jobling, 1981; Kelsch & Neil, 1990). Here, the mean preferred temperature across all treatments (28.9 °C) coincided with the optimal thermal range for aerobic metabolic scope (27-29 °C) and growth (29 °C) for fish used in this study, and the temperature range that has been reported to optimize growth in other tropical coral reef fishes (Zarco-Perello *et al*., 2012, see also Payne *et al.*, 2016).

While a combination of performance metrics, such as aerobic scope, CT_{Max}, and thermal preference may be used to explain broader fitness consequences and population movement patterns, taken alone, each individual metric may not provide enough insight into how an organism will cope with long-term warming. Similar to previous studies, we found a significant increase in CT_{Max} following long-term exposure to elevated temperatures. However, despite the prolonged exposure to 33 °C, *C. viridis* was not able to tolerate rapid or transient heating events. That is, those individuals were not able to recover from the CT_{Max} trials, indicating that long-term exposure to temperatures projected for the near future (33 °C) could prove detrimental, as compensatory mechanisms associated with recovery from heat stress may be exhausted. Consequently, the increase in CT_{Max} only provided a short-term advantage but no clear benefit to the long-term thermal tolerance of individuals. Likewise, the lack of significant change in AMS in fish maintained between 27 °C and 33 °C could lead to the erroneous conclusion that this species is highly thermally tolerant to a 3 °C increase in temperatures, even though critical changes to whole animal fitness may occur in the wild. Specifically, the relatively minor, non-significant, reductions in aerobic scope seen here, may pose a serious physiological threat through the cascading effects of thermal stress on thermoregulatory behaviour (Kearney *et al*., 2009; Sinervo *et al*., 2010; *sensu* Du Plessis *et al*., 2012). That is, while some ectotherms buffer the impacts of elevated temperature by temporarily sheltering in thermal refugia, this requires time and energetic expenses that could have been used for foraging and other important metabolic functions such as growth (Kearney *et al*., 2009; Sinervo *et al*., 2010). Consequently, there may be severe fitness consequences for individuals despite a lack of impact on a single physiological process or metabolic performance measure (Clark *et al*., 2013; Donelson *et al*., 2014).

Although most tropical reef fishes are thought to have some capacity for acclimation and adaptation (Donelson *et al.,* 2011, 2014, Grenchik *et al.,* 2013), any insufficiencies could render many tropical stenotherms vulnerable to temperature changes projected to occur within the next 50-100 years. Several studies have shown improvements in some metabolic performance traits following prolonged acclimation, but this was accompanied by reductions in other fitness attributes, such as reproductive output (Angilletta, 2009; Donelson *et al.*, 2011, 2014). In this study, the critical thermal maxima of *C. viridis* increased proportionally with acclimation temperature, but the associated SMR plateaued at 33 °C. A 10 °C increase in body temperature typically requires 2-3 times more energy (Q_{10} = 2-3, Clarke & Johnston, 1999) in order to maintain standard metabolic rate functions (SMR), and the Q_{10} of 0.97 at 33 °C for *C. viridis* indicates that these fish were unable to fully compensate for the increase in temperature, presumably at a cost to basic maintenance. Accordingly, the body mass of individuals maintained at 33 °C decreased by a staggering 30% during the 27 week trial. Energetic requirements of different organs and tissues vary according to their mass and metabolic requirements (Crnokrak & Roff, 2002; Darveau *et al*., 2002; Crispin & White, 2013) causing tissue-specific physiological processes to contribute differently to the animal's total energetic requirements. For example, 35% of the variation in basal metabolic expenses in some ectotherms can be attributed to differences in heart and liver masses (Garland, 1984). Consequently, even though some tissues/organs keep consuming more energy, denaturation or suppression (Nilsson & Renshaw, 2004; Richards, 2011) of highly O_2 -consuming organs and/or tissue may still lead to a lower mass-adjusted O_2 consumption rate and hence SMR, as observed here. This would be considered a strong sign of unsustainable thermal effects on physiological processes and overall fitness. As a result, the reduction in growth concurrent with inadequate increases in SMR at elevated temperatures may have serious impacts on the overall fitness of this species and be a strong motivation for relocating to more favourable thermal environments.

Ultimately, the potential for successful relocation of coral reef fish species such as *C. viridis*, representing the versatile Pomacentridae family, may depend on their level of coral dependence. Relocation is facilitated or constrained not only by physiologically-mediated thermal preferences, but also by the species' versatility in life history characteristics (Wilson *et al.*, 2010; Figueira & Booth, 2010; Nakamura *et al.,* 2013) such as inter- and intra-specific competition, resource and habitat requirements, and dispersal and/or recruitment potentials (Feary *et al.,* 2013; Harborne, 2013; Nakamura *et al.,* 2013; Seth *et al.*, 2013). For the 90 % of coral reef fish species (and the majority of pomacentrid species) that are only partly dependent of coral reefs (Jones *et al.,* 2004), relocation ultimately depends on each species' level of coral dependence in terms of, for example, food and refugia (Nakamura *et al.*, 2013). Among the pomacentrids, only 16 % of the species are obliged to coral reefs (Jones *et al.,* 2004; Cole *et al.,* 2008; Coker *et al.,* 2014) in terms of habitat (Coker *et al.*, 2014) or feeding (e.g., corallivory; Cole *et al.*, 2008). The remaining pomacentrids are capable of multi-species cohabitation (McCormick & Makey; 1997; Nadler *et al.*, 2016), suggesting redistribution may not only be plausible but also a viable option for these species. Theoretically, obligate coral associated species may be more limited in their capacity for rapid relocation pole-ward (Ben-Tzvi *et al.,* 2008; Feary *et al.,* 2013). However, recent studies have determined that many reef-forming coral species and associated coral reef fishes are also moving toward cooler regions (Yamano et al., 2011; Feary et al., 2013) at rates of up to 14 km year^{-1}, perhaps establishing the foundation for less vagrant species.

The pomacentrid, *C. viridis*, shows little or no capacity for acclimation at the level of aerobic metabolic performance or growth when maintained for extended periods of time to temperatures projected for tropical coral reefs by 2100. Rather, we determined that this species can use movement to behaviourally seek out lower, potentially less thermally-stressful temperatures, those that coincide with present day temperatures from where this fish was collected. Temperature-mediated movement has not previously been evaluated as a primary pathway for coping with rapid ocean warming but is likely to have serious implications for the long-term sustainability of tropical fish populations in their current home ranges, particularly when thermal adaptation is not possible. Critically, the impact of behavioural thermoregulation is likely to be rapidly growing as more and more species are found shifting their distribution ranges toward areas with more hospitable temperatures. Indeed, current evidence suggests that 365 different species across 55 families of tropical fishes are either on the move or have already undergone bio-geographical redistributions or range shifts as a result of climate change and more specifically ocean warming (Figueira & Booth, 2010; Feary *et al*., 2013; Nakamura *et al*., 2013). If our findings for a model coral reef fish species from the species-rich family, Pomacentridae, hold true for other coral reef fishes, then increases in ocean temperatures relative to the thermal preference of species may become the primary driving factor for changing coral reef biodiversity. Tropical coral reef fishes are of global ecological and economical importance (Depczynski *et al*., 2007; Hopkins *et al*., 2011; Cinner, 2014) and require effective management and conservation strategies. However, our capacity to implement these strategies will hinge on our understanding of temperature-mediated changes in physiological performance, resilience, diversity, and distribution over the long-term.

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Figure 1

The effect of treatment temperature on O2 consumption rates as estimates of standard metabolic rates (SMR, green circles; panel (**a**)), maximum metabolic rates (MMR, white circles; panel (**a**)), and aerobic metabolic scope (AMS = MMR-SMR, blue triangles; panel (**b**)) on C. viridis. Values are means ± SEM. Letters demarcate statistically significant differences between fish from the different temperature treatments. NS indicates no significant differences.

Figure 2

(**a**) The effect of treatment temperature on the upper critical thermal limits (CTMax) of C. viridis. The solid triangle indicates the CTMax for fish from the 33 °C treatment temperature; however, no fish from this group survived longer than 24 h following the experiment and were thus excluded from statistical analyses. Values are means ± SEM. Letters demarcate statistically significant differences between fish from the different temperature treatments. (**b**) The effect of treatment temperature on the preferred temperature (Tpref) of C. viridis. The dashed, bold line represents the mean Tpref across all treatments (28.9 °C). The line of equality (dotted line) represents the Tpref if it were to exactly match the treatment temperature. Values are means ± SEM. Letters demarcate statistically significant differences between fish from the different temperature treatments.

Figure 3

Body mass of C. viridis across all temperature treatments prior to experimentation (white bars) and following 27 weeks exposure to treatment temperatures (shaded bars). Values are means ± SEM. Letters demarcate statistically significant differences between fish from the different temperature treatments. Asterisks demarcate differences between body mass at the start of the study and after 27 weeks within each treatment temperature.

933 Figure 1

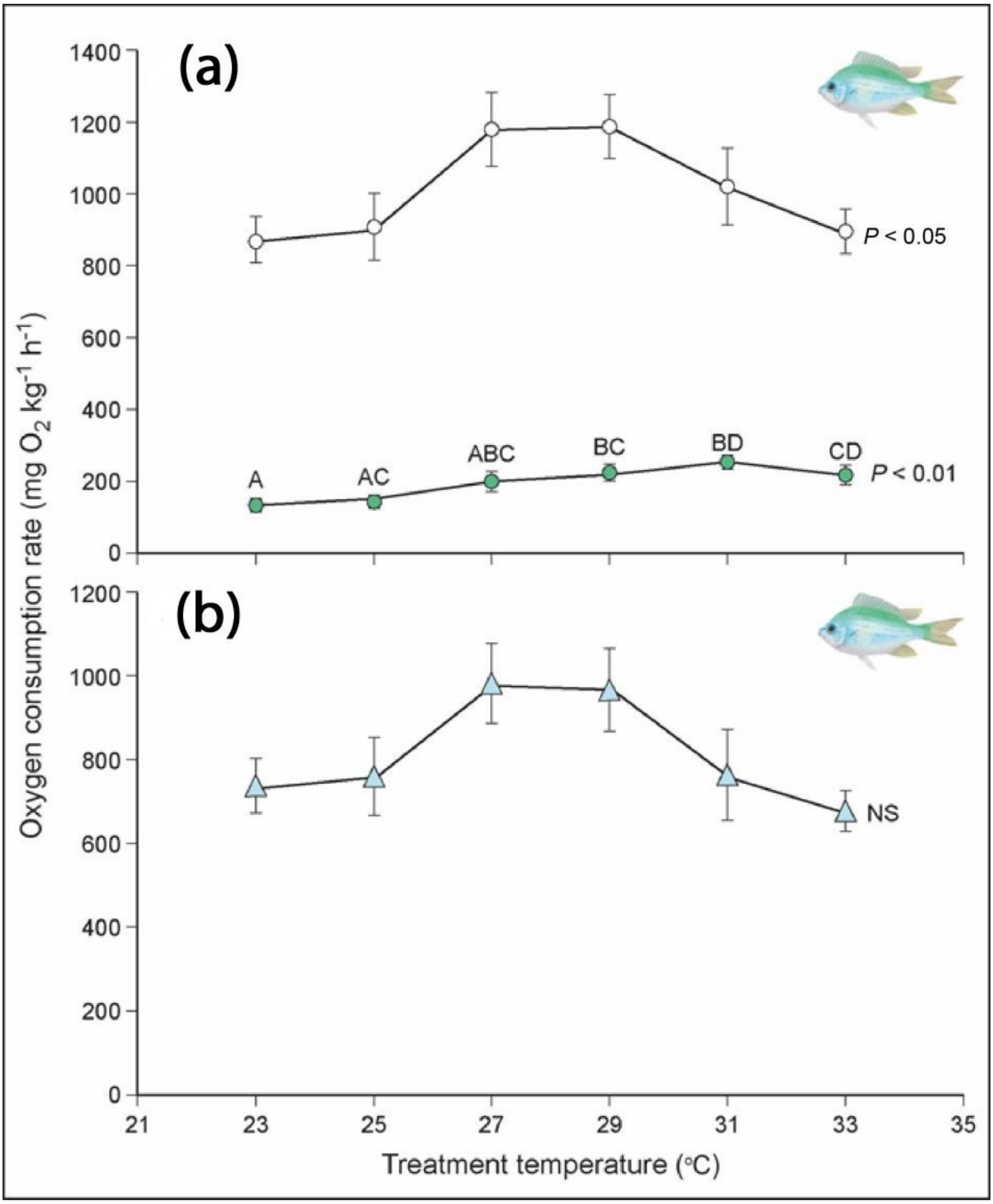

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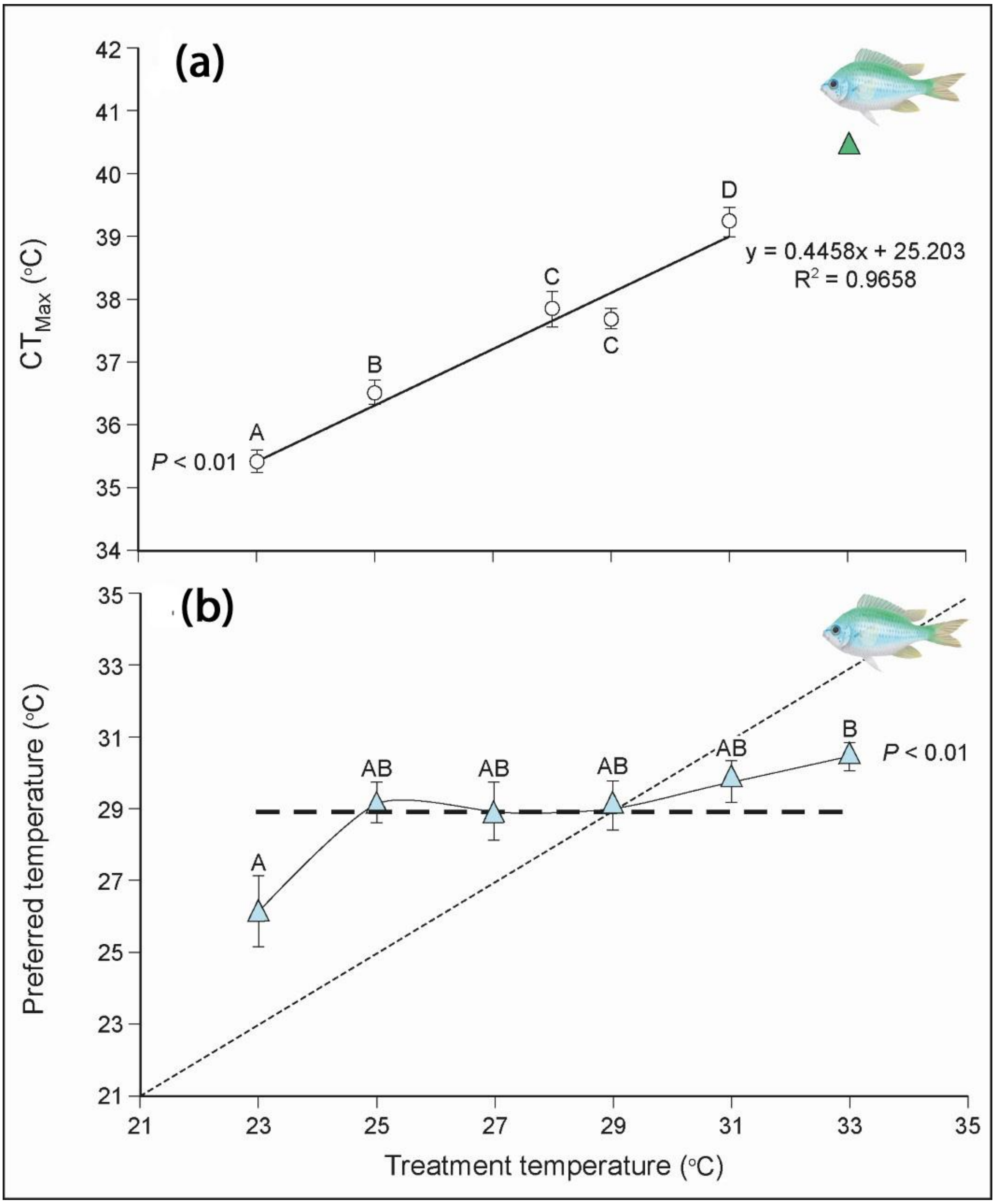
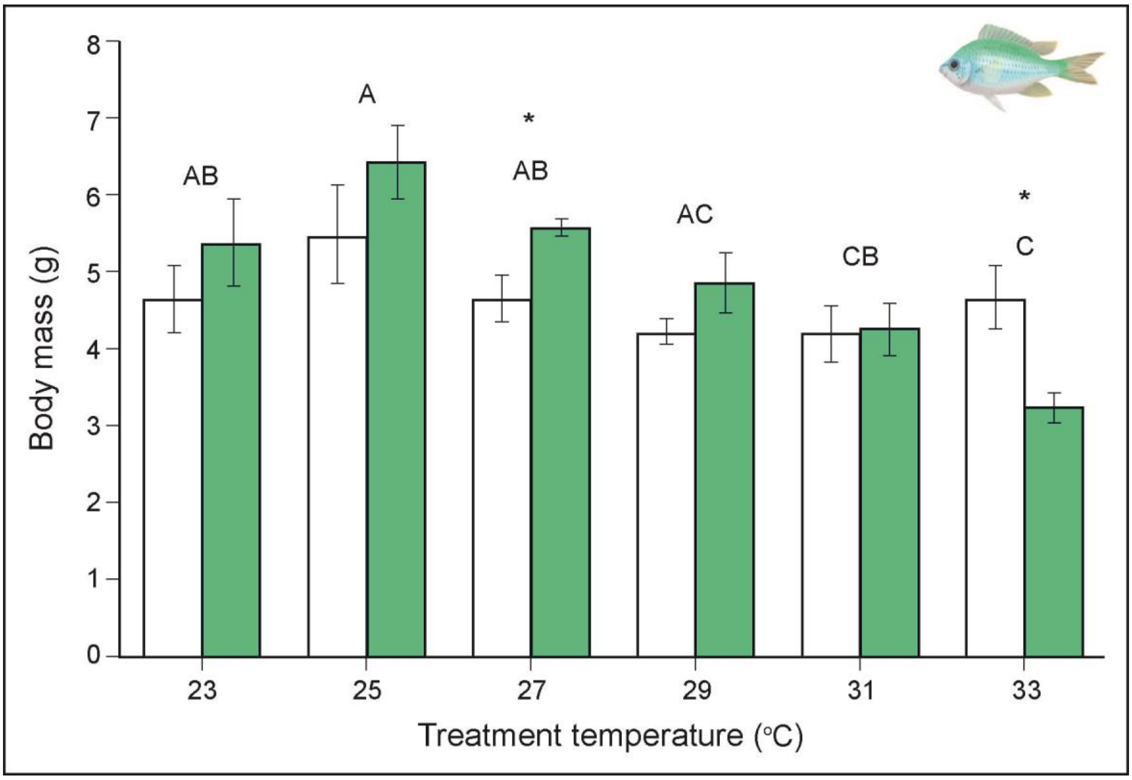
Figure 2


(a)
CT$_{Max}$ (°C)
42
41
40
39
38
37
36
35
34
D
C
C
B
A
P < 0.01
y = 0.4458x + 25.203
R^2 = 0.9658
(b)
Preferred temperature (°C)
35
33
31
29
27
25
23
21
A
AB
AB
AB
AB
B
P < 0.01
21
23
25
27
29
31
33
35
Treatment temperature (°C)

937 Figure 3



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