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

ADAM HABARY1,2, JACOB L. JOHANSEN1,3, TIFFANY J. NAY1, JOHN F. STEFFENSEN2 and JODIE L. RUMMER1

1ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4811, Australia, 2Marine Biological Section, Department of Biology, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark, 3Marine Science Institute, University of Texas at Austin, Port Aransas, TX 78373, USA

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 poleward, 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 6 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 poleward migration of species.

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

Received 30 March 2016; revised version received 17 August 2016 and accepted 17 August 2016

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 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; Gardner 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 6 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; Cumulber & Monks, 2014), acclimation may come at a cost involving 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-Perelló 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 calculated as the difference in oxygen consumption rates ($\bar{V}_O_2$) 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; Osprina & 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; Itikar & Hickey, 2013; Itikar 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 & Neil, 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 & Neil, 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 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 $T_{\text{opt}}$ 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 poleward 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 $T_{\text{opt}}$ and $T_{\text{pref}}$ to projected temperatures.

Focussing on a model species – a thermally sensitive coral reef fish, *Chromis viridis* (Pomacentridae) – we examined the coordinated adjustments of $T_{\text{opt}}$ and $T_{\text{pref}}$ to elevated temperatures expected within general timescales and the propensity of individuals to remain or evade elevated temperatures. We tested three hypotheses: (i) the preferred temperature ($T_{\text{pref}}$) of a tropical stenothermal coral reef fish will coincide with their optimal temperature ($T_{\text{opt}}$) 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 towards 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 damselsfish, *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 12 m or shallower (Randall et al., 1997). This species is highly site-attached, and at all postlarval 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 ± 1.23 g; 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 × 40 cm, height × 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, UT, USA) and provided with multisized shelters in which to hide. Tanks were cleaned on a daily basis. Then, at least 1 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 6 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 24 h to ensure a
postabsorptive 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 aquarium (65 cm × 40 cm: height × diameter) 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 × 4.6 cm; length × 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 s where after, reductions in O2 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 O2 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, 200 L h⁻¹). The measurement period was short enough to ensure that O2 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 PO2. 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 8 h every 24–48 h. Bacterial respiration within the chambers (i.e. the drop in O2 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 MO2 (rest) of the fish. During all trials, each respirometry chamber was partly covered using a cylinder of black plastic with a 2 × 12 cm window. This set-up allowed light to enter the chamber but prevented fish from being disturbed via external visual stimuli.

The O2 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 MO2 in mg O2 kg⁻¹ h⁻¹ at each time point over the 22–24 h 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 & 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- to 24-h recovery period and assigns the peak of the leftmost distribution curve as SMR.

Only slopes with R² ≥ 0.90 were used to reduce the impact of systemic variations in O2 measurements (Svendsen et al., 2016). The proportional background O2 consumption rate (measured as O2 depletion in the empty respirometry chambers before and after each trial, assumed linear) was subtracted from each MO2 measurement. Temperature quotients (Q10) were calculated according to Clarke & 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. CTMax) were determined for 40 randomly selected fish – specifically, eight fish from the 23 °C treatment, eight from 25 °C, seven from 27 °C, six from 29 °C, seven from 31 °C and four from 33 °C – using critical thermal methodology (CTM; Beintinger 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 (Tpref) 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 set-up allows a fish to use movement to control the temperature of its water and therefore its body temperature (i.e., preferred temperature; Tpref). 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 counterclockwise 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 set-up 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 in-line 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 set-up, 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, using position analyser software (LoliTrack, Loli Systems®, Tjelle, 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 Tpref 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 Tpref stabilized within 24 h in all pilot trials and did not change with longer exposure time (paired t-test; P25°C = 0.253, P31°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...
Data analyses

The SMR, MMR, AMS and the effect of body mass were all analysed 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 CTMax data were analysed 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 Tpref values between groups were analysed 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) were analysed 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 log10 transformed for use in models. The level of significance was \( a = 0.05 \) for all tests, and all statistical analyses were performed using STATISTICA v. 12 (Statsoft Inc., Tulsa, OK, USA).

Results

Metabolic rates and aerobic metabolic scope

Treatment temperature did not significantly influence aerobic metabolic scope (AMS) (Fig. 1b; 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 \( \approx 86\% \) from 23 \( ^\circ \)C to 31 \( ^\circ \)C before decreasing again at 33 \( ^\circ \)C (Fig. 1a), and MMR increased by up to 36.4\% between 23 and 29 \( ^\circ \)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 \( ^\circ \)C was 2.06 but fell to 0.97 for SMR estimates between 29 and 33 \( ^\circ \)C and 0.46 between 31 and 33 \( ^\circ \)C.

Fig. 1 The effect of treatment temperature on \( O_2 \) consumption rates as estimates of standard metabolic rates (SMR, shaded circles; panel (a)), maximum metabolic rates (MMR, white circles; panel (a)), and aerobic metabolic scope (AMS = MMR-SMR, shaded triangles; panel (b)) on Chromis viridis. Values are means ± SEM. Letters demarcate statistically significant differences between fish from the different temperature treatments. NS indicates no significant differences.
Critical thermal maxima

There was a significant positive effect of treatment temperature on CT\textsubscript{Max} ($F = 42.55$, $P < 0.01$). Specifically, CT\textsubscript{Max} increased by approximately 0.5 °C for every 1 °C increase in treatment temperature (Fig. 2a, regression equation: $CT_{\text{Max}} = 0.45x + 25.2$, $x = °C$, $R^2 = 0.97$) with average CT\textsubscript{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\textsubscript{Max} of 40.5 °C, but were unable to recover from this exposure and died within 24 h following experimentation. One of the criteria, that is the ability of an animal to escape from conditions that will lead to its death, for CTM (see discussion in Beiting 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 $T_{\text{pref}}$ of $C.\ viridis$ when compared across all treatments (mean $T_{\text{pref}} =$ 28.9 °C, Kruskal–Wallis, $H = 15.7$, $P < 0.01$; Fig. 2b). However, only the $T_{\text{pref}}$ of the 23 °C and 33 °C treatment groups differed from one another ($T_{\text{pref}} =$ 26.1 °C ± 0.96 and 30.4 °C ± 0.37, means ± SEM, respectively, $P = 0.04$; Fig. 2b), and neither the $T_{\text{pref}}$ of fish at 23 °C nor the $T_{\text{pref}}$ of fish at 33 °C were statistically distinct from the $T_{\text{pref}}$ of fish from 25, 27, 29, or 31 °C ($P = 0.31$). The overall mean $T_{\text{pref}}$ for all treatment groups was 28.9 °C, which coincided with the optimum temperature ($T_{\text{opt}}$) 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, $C.\ viridis$ (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 poleward 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
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 6 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 (up to 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-Perelló et al., 2012; see also Payne et al., 2016).

While a combination of performance metrics, such as aerobic scope, CTMax 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 CTMax 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 CTMax 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 CTMax 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, nonsignificant 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 processes.
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 (Q10 = 2–3, Clarke & Johnston, 1999) to maintain standard metabolic rate functions (SMR), and the Q10 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/organisms keep consuming more energy, denaturation or suppression (Nilsson & Rennshaw, 2004; Richards, 2011) of highly O2-consuming organs and/or tissue may still lead to a lower mass-adjusted O2 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 (Figueira & Booth, 2010; Wilson et al., 2010; Nakamura et al., 2013) such as inter- and intraspecific 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 multispecies cohabitation (e.g. McCormick & Makey, 1997; Nadler et al., 2014), 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 poleward (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 towards cooler regions (Yamano et al., 2011; Feary et al., 2013) at rates of up to 14 km yr−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 towards 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.

Acknowledgements

The authors wish to thank the staff of the Marine and Aquatic Research Facilities Unit (MARFU) at James Cook University (JCU) for help with infrastructure and logistical support. Thanks is also due to S. Binning and D. Roche for help with fish collections, G. Collins, S.F. Henriksen and L. Nadler for technical support throughout the experiments, M.B.S. Svendsen for support with data analysis, E. Walsh for fish images and P. Munday for several discussions on this topic. This work was supported by funding from the Generalmajor J.F. Classen, Frøken Ellen Backe from the Generalmajor J.F. Classen, Frøken Ellen Backe and Margaret Munn Tovborg Jensens foundations as well as the Familien Müller-Giehls foundation to A.H. and an ARC Super Science Fellowship and infrastructure and research allocation from the ARC Centre of Excellence for Coral Reef Studies at JCU, both to J.L.R.

References

Donelson JM, Munday PL, McCormick ML (2012b) Climate change may affect fish through an interaction of parental and juvenile environments. Coral Reefs, 31, 753–762.


Hopkins K, Moss BR, Gill AB (2011) Increased ambient temperature alters the parental care behaviour and reproductive success of the three-spined stickleback (Gasterosteus aculeatus). Environmental Biology of Fishes, 90, 121–129.


Seth H, Gräns A, Sandblom E et al. (2013) Metabolic scope and interspecific competition in sculptures of Greenland are influenced by increased temperatures due to climate change. PLoS ONE, 8, e62859.


Steffensen JF (1989) Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiology and Biochemistry, 6, 49–59.


Svensen MBS, Bushnell PG, Christensen EAF, Steffensen JG (2016) Sources of variation in oxygen consumption of aquatic animals demonstrated by simulated constant oxygen consumption and respirometers of different sizes. Journal of Fish Biology, 88, 51–64.


