

RESEARCH ARTICLE

Chronic warm exposure impairs growth performance and reduces thermal safety margins in the common triplefin fish (*Forsterygion lapillum*)

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ABSTRACT

Intertidal fish species face gradual chronic changes in temperature and greater extremes of acute thermal exposure through climate-induced warming. As sea temperatures rise, it has been proposed that whole-animal performance will be impaired through oxygen and capacity limited thermal tolerance [OCLTT; reduced aerobic metabolic scope (MS)] and, on acute exposure to high temperatures, thermal safety margins may be reduced because of constrained acclimation capacity of upper thermal limits. Using the New Zealand triplefin fish (*Forsterygion lapillum*), this study addressed how performance in terms of growth and metabolism (MS) and upper thermal tolerance limits would be affected by chronic exposure to elevated temperature. Growth was measured in fish acclimated (12 weeks) to present and predicted future temperatures and metabolic rates were then determined in fish at acclimation temperatures and with acute thermal ramping. In agreement with the OCLTT hypothesis, chronic exposure to elevated temperature significantly reduced growth performance and MS. However, despite the prospect of impaired growth performance under warmer future summertime conditions, an annual growth model revealed that elevated temperatures may only shift the timing of high growth potential and not the overall annual growth rate. While the upper thermal tolerance (i.e. critical thermal maxima) increased with exposure to warmer temperatures and was associated with depressed metabolic rates during acute thermal ramping, upper thermal tolerance did not differ between present and predicted future summertime temperatures. This suggests that warming may progressively decrease thermal safety margins for hardy generalist species and could limit the available habitat range of intertidal populations.

KEY WORDS: Climate change, Thermal tolerance, OCLTT, Oxygen and capacity limited thermal tolerance, Metabolic scope, Respirometry

INTRODUCTION

Understanding the physiological responses of organisms to temperature change and the limits of thermal tolerance will play a key role in determining a species' susceptibility or resilience to climate change (Somero, 2012). It has recently been suggested that

studies investigating the effects of climate warming may underestimate the impacts on populations if physiological tolerances to environmental extremes are not included, and if only resting physiological functions are considered (Sandblom et al., 2016). The need to examine the impacts of warming on chronic and acute thermal tolerance collectively may also be particularly important in species that occupy habitats that are exposed to large acute temperature fluctuations, such as those within marine intertidal zones (Nilsson and Lefevre, 2016). As the climate warms, these species have to tolerate not only chronic increases in mean ambient sea temperature, but also greater extremes of acute temperature change owing to the forecasted increase in the frequency and severity of transient heat waves (Meehl and Tebaldi, 2004; Perkins et al., 2012). Despite the universal occurrence of rock pools along coastlines, and that predicted changes in climate will likely impact these environments, there is a surprising lack of research regarding both the chronic and acute thermal tolerance of intertidal biota such as fish.

A potential impact of climate change is that chronic warming will demand changes in aerobic metabolism and cause adjustments to essential life history traits such as growth, reproduction, foraging and locomotion, which will lead to changes in fitness (Pörtner and Knust, 2007; Pörtner and Farrell, 2008). This theory, known as oxygen and capacity limited thermal tolerance (OCLTT), has emerged as a prominent hypothesis linking the thermal limits of metabolism to impacts of climate warming on multiple levels of biological organisation in aquatic ectotherms (Clark et al., 2013; Nilsson and Lefevre, 2016; Pörtner, 2010). Under the OCLTT hypothesis, the availability of aerobic metabolic scope (MS; the degree to which an organism can increase oxygen consumption above basal requirement) is the primary determinant and window of a species' thermal tolerance. This thermal window encompasses an optimum temperature where MS peaks (T_{optMS}), upper and lower pejus temperatures that mark the onset of MS decline, and more severe upper and lower critical temperatures where MS is abolished and survival time is limited (Pörtner and Farrell, 2008). In the case of warming, MS is thought to decline at supra-optimal temperatures, because the capacity of the cardiorespiratory system is not capable of increasing maximum oxygen uptake ($\dot{M}_{O_2,max}$) to a rate that keeps pace with increasing basal energetic requirements (Pörtner and Farrell, 2008; Pörtner, 2010). Crucially, it is commonly stated that MS and performance measures such as growth, reproduction and locomotion are causally linked (Claireaux and Lefrancois, 2007; Clark et al., 2013; Lefevre, 2016; Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner, 2010; Schulte, 2015). Despite initial enthusiasm for the OCLTT hypothesis, its general applicability in explaining thermal tolerance and its value in evaluating the potential impacts of climate change have become increasingly questioned (Clark et al., 2013; Nilsson and Lefevre, 2016). A recent meta-analysis

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List of symbols and abbreviations

ATP	adenosine triphosphate
CT _{max}	critical thermal maximum
\dot{M}_{O_2}	mass-specific oxygen consumption
$\dot{M}_{O_2, \max}$	maximum aerobic metabolic rate post-exhaustive exercise
MS	aerobic metabolic scope
OCLTT	oxygen capacity limited thermal tolerance
RMR	routine metabolic rate
RMR _{maxTR}	maximum routine metabolic rate during thermal ramping
SGR	mass-specific growth rate
SMR	standard metabolic rate
SST	sea surface temperature
T_{critMS}	upper critical temperature where metabolic scope is zero
T_{optMS}	temperature optimum for metabolic scope

(Lefevre, 2016) has demonstrated that not all marine ectotherms display the expected ‘increase–optimum–decrease’ temperature response of MS that is a central pillar of the OCLTT hypothesis and that species of interest require assessment on a case-by-case basis to examine the temperature dependence of organismal performance and fitness (Nilsson and Lefevre, 2016). Other studies have also found that the optimum temperature for performance and MS are not always matched (Grans et al., 2014; Norin et al., 2014) and, overall, there are actually few studies linking key performance metrics such as growth to MS under chronic thermal exposures (Clark et al., 2013).

The critical thermal maximum (CT_{max}) is the temperature at which an organism loses equilibrium during acute thermal ramping and is used as a measure of upper thermal tolerance in fish (Beitinger et al., 2000; Mora and Maya, 2006). Fish occupying rock pools may already experience acute exposure to temperatures exceeding critical limits, and climate change will likely increase temperature extremes further as heat waves are predicted to become more extreme (Perkins et al., 2012). In fish, warm acclimation can elevate upper thermal tolerance (Beitinger et al., 2000; Drost et al., 2016; Fangue et al., 2006), which indicates that species’ physiological plasticity may buffer thermal limits as the climate warms. However, evidence suggests that this capacity to elevate the upper thermal tolerance point is likely limited in the context of predicted temperature rises associated with climate change (Gunderson and Stillman, 2015). Moreover, the increase in CT_{max} with warm acclimation so far has always been less than the difference between two acclimation temperatures (Gunderson and Stillman, 2015). Consequently, thermal safety margins (the difference between acclimation temperature and CT_{max}) are reduced with warming (Sandblom et al., 2016). Although rock pools are among the most likely habitats where the CT_{max} of fish is exceeded (and this risk increases with climate change), we are unaware of any study that specifically addresses the acclimation capacity of CT_{max} in intertidal fish in the context of climate change.

The upper critical temperature (T_{critMS}), not to be confused with CT_{max}, is incorporated into the OCLTT hypothesis as the temperature at which MS becomes zero (Pörtner, 2010). Because warm acclimation increases the upper thermal tolerance of fish, this should also be associated with metabolic adjustments, which increase or maintain MS at high temperatures. Indeed, under the OCLTT hypothesis, improved upper thermal tolerance in warm-acclimated organisms is proposed to result from downregulation of mitochondrial function, which decreases resting oxygen demand and protects MS during warming (Pörtner, 2001). Conversely, cold-acclimated organisms have higher mitochondrial densities to

maintain capacity for MS and adenosine triphosphate (ATP) production under cold temperatures, but this also increases oxygen demand during warming and decreases upper thermal tolerance (Fangue et al., 2009; Pörtner, 2001). Thus, at an equivalent elevated temperature, a thermally tolerant warm-acclimated ectotherm would have a lower metabolic rate than a less tolerant individual acclimated to cooler temperatures (Fangue et al., 2009).

Forsterygion lapillum Hardy 1989 is a small (20–60 mm) benthic marine blennioid fish that occupies shallow subtidal reefs, harbours and intertidal rock pools of New Zealand. This species maintains MS across a thermal acclimation range of 15–25°C and shows a surprisingly high T_{optMS} of 24°C (Khan et al., 2014a). Under the OCLTT framework, a T_{optMS} of 24°C would indicate that whole-animal performance may be optimised at a temperature beyond the present-day range, but this seems questionable because significant mortality (50%) occurs upon acclimation to 25°C, and skeletal muscle mitochondrial ATP production capacity shows signs of compromise at 24°C (Khan et al., 2014a). As an in-depth understanding of temperature-specific performance is not known for this species, the possibility of optimised performance at temperatures beyond present-day conditions remains unclear.

As *F. lapillum* occupies intertidal rock pools and shallow subtidal habitats, this study set out to critically evaluate the chronic and acute thermal tolerance of *F. lapillum* after 12 weeks of thermal acclimation to 15, 18, 21 and 24°C. To critically evaluate the growth performance and resilience of *F. lapillum* to climate change, the mass-specific growth rate (SGR) was first measured at the four acclimation temperatures. It was hypothesised that the fitness performance of the 24°C group [i.e. fish chronically exposed to temperatures ~3°C above the current mean peak summer sea surface temperature (SST) in northern New Zealand] would be negatively impacted. As part of this testing, MS at the four temperatures was also measured and compared against growth to determine whether growth performance and metabolic performance correlated across an extended time frame as expected under the OCLTT framework. Combining archival SST records with the temperature–growth data of *F. lapillum* then provided the opportunity to model the seasonal growth impact of future warming scenarios within the northern geographical range of its distribution. Following the growth trial, the acute thermal tolerance (CT_{max}) of thermally acclimated *F. lapillum* (15, 18, 21 and 24°C) was then assessed to gauge whether plasticity in upper thermal tolerance exerts any influence over the ability of *F. lapillum* to withstand short-term extremes in temperature during a thermal ramping test. We hypothesised that higher acclimation temperatures would increase upper thermal tolerance (Beitinger et al., 2000) and that this would be associated with a lower metabolic rate on thermal ramping, as predicted by the OCLTT hypothesis (Fangue et al., 2009).

MATERIALS AND METHODS**Experimental animals**

Wild *F. lapillum* were caught using bait catcher traps and transferred to the Leigh Marine Laboratory (Leigh, New Zealand), where they were housed in 30 l flow-through ambient temperature seawater tanks (14.7±0.16°C, air saturated, 200 µm filtered, 35 ppt salinity) for a period of 14 days to allow laboratory acclimation. These fish had an initial total mean (±s.e.m.) length of 55.3±0.55 mm and mass of 1.53±0.03 g and were sexually mature 1- to 2-year-old fish based on length-at-age determinations for this species (P. E. Caiger, unpublished). During this acclimation period they were fed daily to satiation on a mixture of crushed aquaculture pellets (Skretting,

Australia) and chopped pilchard. Animal ethics approval was granted from The University of Auckland Animal Ethics Committee (AEC approval 001801).

Growth trial

For the duration of the growth trial the fish were housed in 15 l flow-through seawater tanks (air saturated, 200 μm filtered, 35 ppt salinity) maintained at the target experimental temperatures of 15, 18, 21 or 24°C ($\pm 0.3^\circ\text{C}$). The temperature was maintained in each tank by continually heating or cooling water to the required temperature with heat pumps. Each heat pump received seawater from insulated tanks, which were continuously fed with fresh ambient temperature seawater (200 μm filtered, 35 ppt salinity) from the main laboratory supply. Three replicate tanks were each randomly assigned eight fish at the four temperatures ($N=24$ fish per temperature). Each individual was tagged with visible subcutaneous elastomer (NMT INC Northwest Marine Technology, USA) and allowed to recover over 24 h at 15°C without feeding. The initial mass and length of each fish was then measured and the temperature of each tank was raised to the target experimental temperature at a rate of 3°C per 24 h. Mass-specific growth rates (SGR; % body mass day^{-1}) of individual fish were then assessed at 30 day intervals over a 12 week period according to:

$$\text{SGR} = \ln m_2 - \ln m_1 / t_2 - t_1 \times 100, \quad (1)$$

where m_1 is the initial mass at the start of the growth period t_1 and m_2 is the final mass at the end of the growth period t_2 . The overall growth rate of individual fish was taken as the mean SGR of the three 30 day growth periods. Throughout the growth trial each tank was fed daily to satiation on a mixture of crushed aquaculture feed (Skretting) and chopped pilchard. Ammonia, nitrate and nitrite were regularly measured in each tank and water quality remained high throughout the trial.

Respirometry

At the conclusion of the growth trial, metabolic rate and acute thermal tolerance were then assessed in all individuals. Metabolic and thermal tolerance measurements were carried out in an automated respirometry set-up that could monitor four fish simultaneously in individual chambers (~ 20 h per run). All trials were completed over 35 days and, to avoid confounding handling effects on metabolic measurements, growth measurements were not assessed during this period. The temperature treatment for each set of four fish was assessed in a cycled order so that the period between the conclusion of the 12 week growth trial and assessment of metabolic rate and upper thermal tolerance was as similar as possible across treatment groups.

The mass-specific O_2 consumption rate (\dot{M}_{O_2}), reported as milligrams of oxygen consumed per gram of body mass per hour ($\text{mg O}_2 \text{g}^{-1} \text{h}^{-1}$), was determined using automated intermittent-flow respirometry (Steffensen, 1989). The inlet of each chamber was connected to an automated Eheim compact 3000 submersible flush pump (EHEIM GmbH & Co. KG, Germany) that was switched on and off by a relay control unit (USB Power 8800 Pro, Aviosys International Inc., Taiwan) controlled by custom-coded software (Leigh Marine Laboratory). A magnetic stir bar was housed in a recess in the bottom of the respirometry chamber to ensure adequate water mixing and the oxygen concentration of water within the chamber was continuously measured using contactless sensor spots followed by FireSting O_2 meters (PyroScience, Aachen, Germany). The decline in O_2 concentration within a respirometry chamber was

used to calculate \dot{M}_{O_2} in repeated measurement cycles (4–8 min) according to the equation:

$$\dot{M}_{\text{O}_2} = V \left(\Delta\% \frac{S}{t} \right) \alpha M_b, \quad (2)$$

where V is the respirometry chamber minus fish volume, $\Delta\%S/t$ is the change in percentage air saturation of oxygen per unit time, α is the solubility coefficient of oxygen ($\text{mg O}_2 \text{ \%S}^{-1} \text{ l}^{-1}$) in seawater (35 ppt) and M_b is the body mass of the fish in grams (Schurmann and Steffensen, 1997). The repeated measurement cycles were interspersed by automated flushing periods (1 min) so that the water within the chamber was refreshed and maintained at an O_2 concentration above 80% air saturation.

Metabolic measurements were completed in a sequence where $\dot{M}_{\text{O}_2, \text{max}}$ was assessed after exhaustive exercise; this was then followed by measures of standard metabolic rate (SMR) during an overnight period of respirometry. $\dot{M}_{\text{O}_2, \text{max}}$ was determined following exhaustive exercise where fish were continually chased by hand for a period of 5 min. The chasing was carried out in a 30 l tank filled with air-saturated seawater maintained at the required experimental temperature. The fish were visibly exhausted after chasing and this method was used as it has been previously used to elicit maximal \dot{M}_{O_2} values in *F. lapillum* (Khan et al., 2014a) and is the most reliable method for obtaining $\dot{M}_{\text{O}_2, \text{max}}$ in benthic species that do not swim continuously in a flume (Clark et al., 2013; Norin and Clark, 2016). The fish were then transferred to a respirometry chamber within 30 s of the conclusion of chasing and repeating 4 min \dot{M}_{O_2} measurement cycles were initiated. $\dot{M}_{\text{O}_2, \text{max}}$ was taken as the highest \dot{M}_{O_2} value recorded in any measurement cycle, which in almost all cases was obtained from the first measurement cycle following exhaustive exercise. When the metabolic rate of fish was clearly declining, the measurement period was extended to 8 min and \dot{M}_{O_2} was measured repeatedly for >16 h. During overnight \dot{M}_{O_2} measurements, fish were left undisturbed in the respirometers and SMR was estimated from the mean of the lowest 10% of \dot{M}_{O_2} over this time (Khan et al., 2014a; Norin et al., 2014). MS was defined as the difference between the body-mass-corrected $\dot{M}_{\text{O}_2, \text{max}}$ and SMR (see below) for each individual. In all estimates of metabolic rate, only \dot{M}_{O_2} values with $R^2 > 0.95$ for the decline in oxygen per unit of time were used.

Acute thermal tolerance and critical thermal maximum

Following SMR estimation, fish were subjected to thermal ramping to determine CT_{max} . In all cases, thermal ramping for an individual fish began from the experimental temperature a particular fish had been acclimated to in the growth trial phase of the study. The temperature of the seawater reservoir was increased at a rate of 4°C h^{-1} and water temperature was continuously measured using a shielded temperature probe connected to a FireSting O_2 system (PyroScience, Aachen, Germany). After every 1°C increment in temperature, the routine \dot{M}_{O_2} (RMR) of fish was measured over a measurement cycle length of 3–5 min. Thermal ramping and \dot{M}_{O_2} measurements continued until the point at which fish lost equilibrium for 10 s or more, which was taken as the CT_{max} (Beitinger et al., 2000; Brijis et al., 2015; Sandblom et al., 2016). The fish was then removed from the respirometer and transferred to air-saturated ambient temperature seawater ($\sim 21^\circ\text{C}$) for recovery. The maximum routine metabolic rate during thermal ramping ($\text{RMR}_{\text{maxTR}}$) was taken as the highest \dot{M}_{O_2} measurement obtained at any of the 1°C temperature increments prior to reaching the point of CT_{max} . The thermal safety margin for each fish was

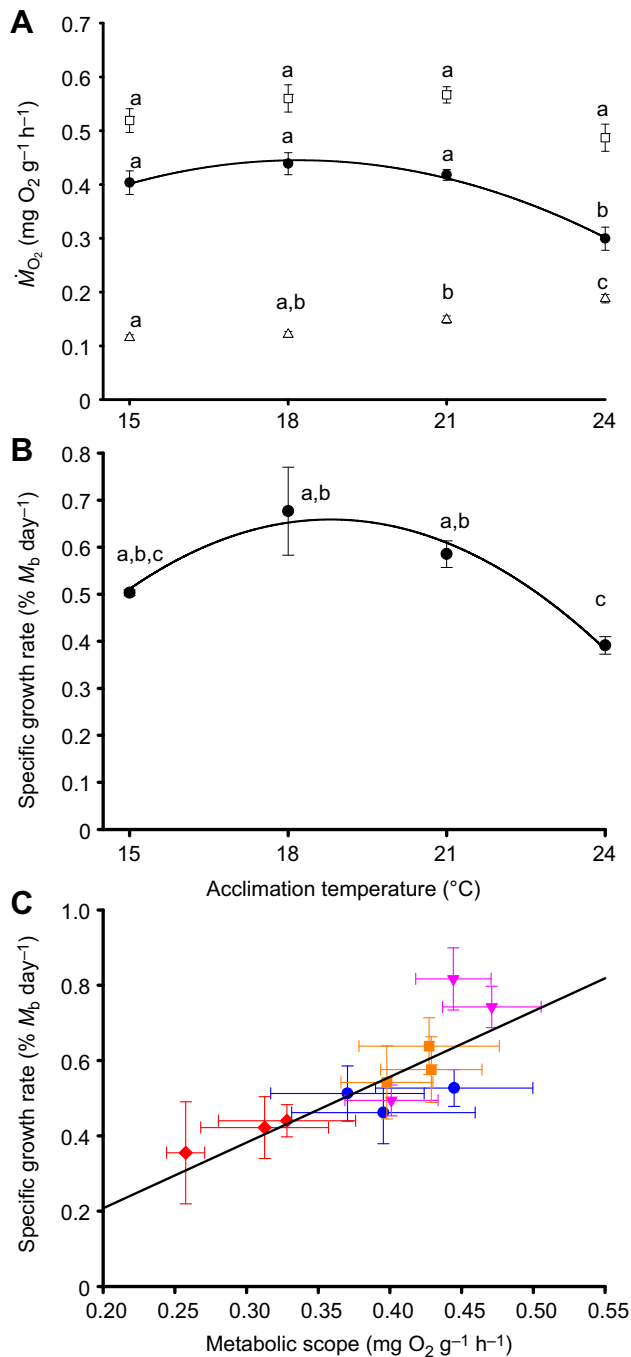


Fig. 1. Metabolic rate and growth responses of *Forsterygion lapillum* acclimated to 15, 18, 21 and 24°C. (A) Mean (\pm s.e.m.) standard metabolic rate (open triangles), maximum metabolic rate (open squares) and aerobic metabolic scope (closed circles) ($N=3$ tank replicates for each temperature, $N=18$ –23 fish per temperature). Significant differences (one-way ANOVA, $P<0.05$) are shown for each of standard metabolic rate, maximum metabolic rate and aerobic metabolic scope by lowercase letters that are not common between acclimation temperatures. The black line shows a polynomial (quadratic) regression ($y=-0.0043x^2+0.1556x-0.9709$, $R^2=0.79$, $P=0.0008$) between acclimation temperature and aerobic metabolic scope. (B) Mean (\pm s.e.m.) specific growth rate ($N=3$ tank replicates for each temperature, $N=20$ –23 fish per temperature). Significant differences ($P<0.05$) are shown by lowercase letters that are not common between temperatures. The black line shows a polynomial (quadratic) regression ($y-0.0102x^2+0.3837x-2.9479$, $R^2=0.67$, $P=0.0069$) between temperature and specific growth rate. M_b , body mass. (C) Mean (\pm s.e.m.) specific growth rate and aerobic metabolic scope of individual tank replicates (blue circles=15°C, pink triangles=18°C, orange squares=21°C, red diamonds=24°C, $N=3$ tank replicates for each temperature). The black line shows a linear regression between specific growth rate and aerobic metabolic scope ($R^2=0.69$, $P<0.001$).

Scaling of metabolic measurements and statistics

To account for body mass differences, the \dot{M}_{O_2} values were standardised to the mean body mass of all fish (2.5 g) using the formula:

$$\dot{M}_{O_2,2.5g} = \dot{M}_{O_2,meas} \left(\frac{m}{m_{2.5g}} \right)^{(1-A)}, \quad (3)$$

where $\dot{M}_{O_2,2.5g}$ is the \dot{M}_{O_2} for a fish with the standardised (corrected) new mass of 2.5 g, $\dot{M}_{O_2,meas}$ is the measured \dot{M}_{O_2} , m is the mass of the fish, $m_{2.5g}$ is the standardised body mass of fish set to 2.5 g and A is the mass exponent describing the relationship between metabolic rate and body mass (Schurmann and Steffensen, 1997). The mass exponents (A) describing the relationship between body mass and \dot{M}_{O_2} for SMR and $\dot{M}_{O_2,max}$ were 0.75 and 0.65, respectively, and were derived from a dataset of triplefin \dot{M}_{O_2} measurements collected across multiple studies (T.J.M., unpublished data). The values for these mass exponents are within the expected range for fish (Clarke and Johnston, 1999).

In all statistical tests, significance was accepted at $P<0.05$. Mixed-model analysis was used to perform a nested analysis of covariance and assess the effect of acclimation temperature on growth rate (SGR) following the model fitting procedure outlined by Zuur et al. (2009). The final model included initial fish mass as a covariate, temperature as a fixed main effect and, to control for possible tank effects, individual tanks as fixed random effects. The model assumptions of normality and homoscedasticity were satisfied and specific *post hoc* comparisons were carried out using Tukey contrasts. The relationship between SGR and temperature was also assessed with a second-order (non-linear) polynomial regression. To examine how climate warming may impact annual patterns of growth, the relationship between temperature and SGR derived from the laboratory-based growth trial (Fig. 1B) was used to calculate projected growth rates over an annual present-day temperature regime and annual temperature regimes based on two warming scenarios (present-day temperature +1.5 and +3°C). The annual present-day temperature regime was based on the 7 day temperature means from a 40 year SST record from the Leigh Marine Laboratory, which is in the northeastern region of this species' geographical range and where the *F. lapillum* used in this study were sourced. SMR, $\dot{M}_{O_2,max}$, MS, CT_{max} , thermal safety margin and RMR_{maxTR} were each compared between acclimation temperatures using one-way ANOVA with Holm–Sidak *post hoc*

also calculated and was defined as the temperature difference between CT_{max} and the acclimation temperature (Sandblom et al., 2016).

Background oxygen consumption was measured immediately after thermal ramping. Pilot trials showed that background oxygen consumption remained negligible throughout the overnight SMR measurement cycles but developed linearly during thermal ramping. A linear regression was therefore used to back-calculate the background oxygen consumption values throughout thermal ramping and these were subtracted from fish \dot{M}_{O_2} . The mean background oxygen consumption at 15, 18, 21 and 24°C was 3.2 ± 0.72 , 3.4 ± 0.5 , 3.9 ± 0.64 and $4.7 \pm 0.6\%$ of fish \dot{M}_{O_2} during thermal ramping, respectively.

comparisons and individual tanks treated as replicates ($N=3$ per temperature). The relationship between MS and temperature was also assessed with a second-order (non-linear) polynomial regression and linear regression was used to examine the relationship between SGR and MS across tank replicates. RMR during thermal ramping was compared between temperature acclimation treatments using two-way repeated-measures ANOVA with individual tanks set as subjects. Thermal ramping for each temperature acclimation treatment started at a different temperature so RMR was compared over a standardised temperature range of 24 to 28°C. This temperature range was chosen as it was common to all four acclimation temperatures and did not include temperatures where any fish had obviously declining \dot{M}_{O_2} , or had lost equilibrium. Finally, the relationship between RMR at 24°C and CT_{max} and RMR at 28°C and CT_{max} was examined using linear regression. All statistical tests were carried out using R (version 3.1.1) and Sigma Plot (Systat Software, CA, USA).

RESULTS

Mortality

There were nine mortalities during the 12 week growth trial but there were no significant differences in mortality rate between the temperature acclimation treatments ($\chi^2=2.33$, d.f.=3, $P=0.507$). The surviving fish appeared in good health at all temperatures with no obvious signs of disease or abnormal behaviour indicative of a compromised health status.

Chronic thermal tolerance and the relationship between growth rate, metabolic rate and temperature

SMR was significantly different between the temperature acclimation treatments (ANOVA, d.f.=3, $F=20.2$, $P<0.001$) and there was an overall trend of SMR to increase with higher temperature (Fig. 1A). *Post hoc* tests showed that SMR was significantly higher at 24°C than at 21°C ($P=0.027$), 18°C ($P=0.002$) and 15°C ($P<0.001$), and significantly higher at 21°C compared with 15°C ($P=0.024$). $\dot{M}_{O_{2,max}}$ appeared lowest in fish acclimated to 24°C but no

significant differences (ANOVA, d.f.=3, $F=2.79$, $P=0.11$) were found between temperature treatment groups (Fig. 1A). A parabolic relationship described the effect of acclimation temperature on MS, with MS peaking at 18.2°C (Fig. 1A). There was a significant main effect of temperature on MS (ANOVA, d.f.=3, $F=10.58$, $P=0.004$) and *post hoc* tests showed significantly lower MS at 24°C than at 15°C ($P=0.019$), 18°C ($P=0.005$) and 21°C ($P=0.011$). Fish held at all temperatures gained mass during the 12 week temperature acclimation period and there was a parabolic relationship between SGR and temperature, with the highest growth rate seen at 18°C ($0.68 \pm 0.041\% M_b \text{ day}^{-1}$) but lower growth rates at 21°C ($0.59 \pm 0.047\% M_b \text{ day}^{-1}$), 15°C ($0.51 \pm 0.034\% M_b \text{ day}^{-1}$) and 24°C ($0.39 \pm 0.048\% M_b \text{ day}^{-1}$; Fig. 1B). Mixed-model analysis confirmed a significant main effect of temperature (d.f.=3, $F=5.28$, $P=0.027$) on SGR and *post hoc* comparisons showed SGR was significantly higher at both 18°C ($P<0.001$) and 21°C ($P=0.041$) compared with 24°C. The similar pattern in SGR and MS across the temperature acclimation treatments resulted in a significant linear relationship between these variables ($R^2=0.48$, $P=0.01$), with an overall trend for higher SGR to be associated with greater MS across the individual tank replicates (Fig. 1C).

Projected annual patterns of growth under present-day and future (warming) temperature regimes

Under a present-day annual temperature regime, optimum temperatures for growth in *F. lapillum* occur over the warmer summer and early autumn months between December and May but growth potential drops substantially over the cooler winter period between June and November (Fig. 2). Under a scenario where the temperature at all times of the year is 3°C warmer than present day, optimum temperatures for growth occur over late autumn, winter and spring (May to November), whereas growth potential declines substantially over the warm summer months (Fig. 2). Despite substantial changes in the projected annual pattern of growth under warming scenarios, only minor differences are projected in the mean annual growth rate of *F. lapillum*, where a small but possibly

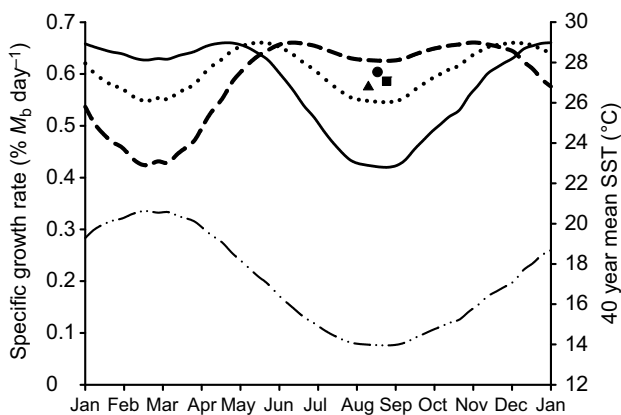


Fig. 2. Projected annual cycle of growth performance in *Forsterygion lapillum* under present-day and future (warming) temperature regimes. All values of projected specific growth rate (left axis) are calculated from a significant non-linear relationship between growth rate and temperature described by the equation $y = -0.0102x^2 + 0.3837x - 2.9479$ (Fig. 1B), where y is specific growth rate (% M_b per day) and x is temperature (°C). 40 year mean sea surface temperature (SST) from the Leigh Marine Laboratory site (dotted and dashed line, right axis) was used to calculate projected specific growth rate throughout the year for 40 year mean SST (solid line), 40 year mean SST+1.5°C (dotted line) and 40 year mean SST+3°C (dashed line). Symbols represent the mean annual projected growth rate for 40 year mean SST (triangle), 40 year mean SST+1.5°C (circle) and 40 year mean SST+3°C (square).

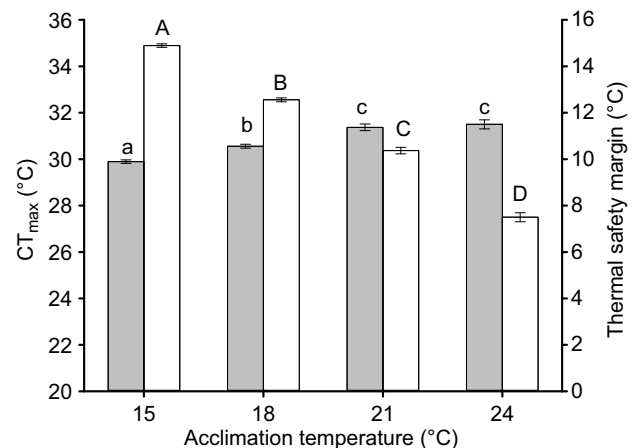


Fig. 3. Upper thermal tolerance in *Forsterygion lapillum* acclimated to 15, 18, 21 and 24°C. Grey and white bars show the mean (\pm s.e.m.) critical thermal maximum (CT_{max} , left-hand axis) and thermal safety margin (right-hand axis), respectively ($N=3$ tank replicates for each temperature, $N=18-23$ fish per temperature). The thermal safety margin is the difference between acclimation temperature and critical thermal maximum temperature. Dissimilar lowercase and uppercase letters above bars indicate significant differences (one-way ANOVA, $P<0.05$) between acclimation temperatures for CT_{max} and thermal safety margin, respectively.

insignificant increase exists compared with present day under +1.5 and +3°C warming scenarios (Fig. 2).

Acute thermal tolerance

Following thermal acclimation, CT_{max} was highest in fish acclimated to 24°C and lowest in those acclimated to 15°C, and there was a significant main effect of acclimation temperature (ANOVA, d.f.=3, $F=32.39$, $P<0.001$; Fig. 3). *Post hoc* tests showed that fish acclimated to 24 and 21°C had significantly higher CT_{max} than fish acclimated to 18°C ($P=0.004$, $P=0.007$) and 15°C ($P<0.001$, $P<0.001$) and fish acclimated to 18°C had significantly higher CT_{max} than fish acclimated to 15°C ($P=0.015$). The difference between acclimation temperature and CT_{max} (thermal

safety margin) was progressively reduced with increasing acclimation temperature and there were significant differences in thermal safety margin between all temperature acclimation treatments (ANOVA, d.f.=3, $F=570.87$, $P<0.001$; Fig. 3).

Metabolic rate during thermal ramping

During thermal ramping, the RMR of fish acclimated to all temperatures increased to a peak in an exponential manner and then declined suddenly (Fig. 4A). Across a common temperature range (24–28°C) there was a significant effect of acclimation temperature (ANOVA, d.f.=3, $F=17.13$, $P<0.001$) and thermal ramping temperature (ANOVA, d.f.=4, $F=195.6$, $P<0.001$) on RMR and the interaction was significant (ANOVA, d.f.=12, $F=2.59$, $P=0.016$). *Post hoc* tests showed that RMR increased significantly ($P<0.05$) between 24 and 28°C, but there were also significant differences between acclimation temperatures that were dependent on thermal ramping temperature. Between 24 and 26°C, RMR was significantly higher in fish acclimated to 15 and 18°C than in fish acclimated to 21 and 24°C (Fig. 4A). However, at 27 and 28°C, fish acclimated to 24°C had significantly lower RMR than those acclimated to 15, 18 and 21°C, and those acclimated to 21°C had significantly lower RMR than 15°C acclimated fish (Fig. 4A). RMR attained a similar maximal \dot{M}_{O_2} value in the 15°C ($0.58 \text{ mg O}_2 \text{ g}^{-1} \text{ h}^{-1} \pm 0.028$), 18°C ($0.59 \text{ mg O}_2 \text{ g}^{-1} \text{ h}^{-1} \pm 0.006$) and 21°C ($0.56 \text{ mg O}_2 \text{ g}^{-1} \text{ h}^{-1} \pm 0.021$) groups but appeared to increase less in the 24°C group ($0.49 \text{ mg O}_2 \text{ g}^{-1} \text{ h}^{-1} \pm 0.015$; Fig. 4A,B). As a result, peak values of RMR during thermal ramping were significantly different between the temperature acclimation groups (ANOVA, d.f.=3, $F=4.98$, $P=0.031$), and *post hoc* tests confirmed that fish acclimated to 24°C had significantly lower RMR_{maxTR} than those acclimated to 18°C ($P=0.043$; Fig. 4B). Interestingly, there was a significant inverse linear relationship between RMR and CT_{max} at 24°C ($R^2=0.67$, $P=0.001$) and also between RMR and CT_{max} at 28°C ($R^2=0.48$, $P=0.013$). As such,

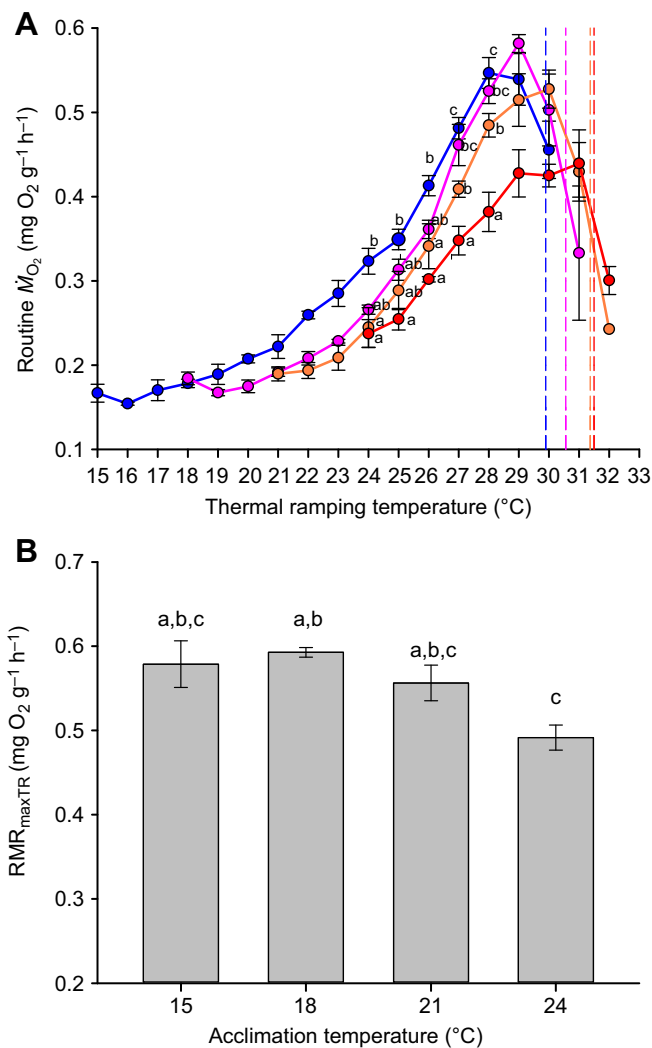


Fig. 4. Routine metabolic rate during acute thermal ramping in *Forsterygion lapillum* acclimated to 15, 18, 21 and 24°C. (A) Routine metabolic rate (\dot{M}_{O_2}) during thermal ramping. Data points represent the mean (\pm s.e.m.) of tank replicates ($N=3$ tank replicates for each acclimation temperature, $N=18$ –23 fish per temperature). Blue, 15°C; pink, 18°C; orange, 21°C; red, 24°C. Dashed vertical lines represents the mean critical thermal maximum for reference (i.e. data from Fig. 3). Lowercase letters not shared between data points show significant differences ($P<0.05$) in routine metabolic rate among temperature acclimation treatments at thermal ramping temperatures between 24 and 28°C. (B) Maximum routine \dot{M}_{O_2} during thermal ramping (RMR_{maxTR}). Data points represent the mean (\pm s.e.m.) of tank replicates. Lowercase letters not shared between data points show significant differences (one-way ANOVA, $P<0.05$).

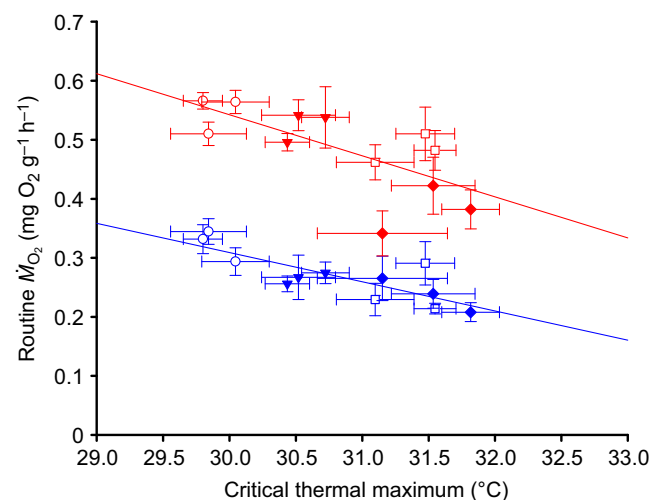


Fig. 5. The relationship between routine metabolic rate during thermal ramping and critical thermal maximum in *Forsterygion lapillum* acclimated to 15, 18, 21 and 24°C. Data points represent mean (\pm s.e.m.) routine \dot{M}_{O_2} at 24°C (blue symbols) and 28°C (red symbols), and mean (\pm s.e.m.) critical thermal maximum of each tank replicate (open circles, 15°C; closed triangles, 18°C; open squares, 21°C; closed diamonds, 24°C; $N=3$ tank replicates for each temperature). The blue line shows a linear regression between routine \dot{M}_{O_2} at 24°C and critical thermal maximum ($R^2=0.67$, $P<0.01$) and the red line shows a linear regression between routine \dot{M}_{O_2} at 28°C and critical thermal maximum ($R^2=0.48$, $P<0.05$).

low RMR was consistently associated with a high CT_{max} and vice versa (Fig. 5).

DISCUSSION

To better understand how global warming may affect fish populations and their ecosystems, previous research has stimulated the need for more data on the response of respiratory variables, fitness-related variables and upper thermal tolerance limits to warming in species of interest (Nilsson and Lefevre, 2016). In this respect, the current research has adopted a novel approach in addressing growth and metabolic responses of the common triplefin fish to chronic and acute thermal changes. This provides insights into possible impacts of climate change for a highly abundant coastal species that resides in shallow subtidal and intertidal habitats. This work also allows an opportunity to assess the relevance of the OCLTT hypothesis in chronic and acute thermal tolerance.

Impact of chronic warm exposure on growth performance and metabolic scope

There are few studies that allow for the potential effects of thermal acclimation of metabolism on MS under chronic time scales (weeks to years) (Norin et al., 2014; Sandblom et al., 2014, 2016) and even fewer that include measurements of growth (Donelson, 2015; Grans et al., 2014; Khan et al., 2014b). In the warm northern geographical range of *F. lapillum*, mean SST peaks in February (20.6°C Leigh Laboratory SST record) and remains within 0.5°C of this value for a period of 11 weeks over the austral summer. Thus tolerance assessments at higher mean temperatures approximating future temperature predictions should be tested over a similar time frame. This justifies our 12 week period of continuous temperature acclimation.

Chronic exposure to 24°C resulted in decreased growth rates and MS compared with lower temperatures (15, 18 and 21°C). Our data therefore indicate that an average sea temperature rise to 24°C owing to climate change will impair the whole-animal and metabolic performance of this species during the hottest months of the year. Depressed MS at 24°C was driven by higher SMR, and a marginally decreased $\dot{M}_{O_2,max}$ in comparison to fish acclimated to lower temperatures. The metabolic response of *F. lapillum* to chronic warming therefore follows the response pattern outlined in the OCLTT hypothesis, where maximum aerobic capacity is limited, and warming beyond an optimal temperature increases basal costs and reduces MS (Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner, 2010). However, this pattern of restricted MS at higher temperature is not universal across species, as the MS of some fish increases continuously with temperature (i.e. it does not peak) (Lefevre, 2016), because of the expansion of $\dot{M}_{O_2,max}$ at higher temperatures. If MS theoretically sets a limit to the extent to which aerobically demanding processes can be performed beyond maintenance requirements (Clark et al., 2013), it is perhaps not surprising that the growth of *F. lapillum* fell at higher temperatures. MS was decreased and competing oxygen-demanding processes were potentially partitioned or prioritised across a shrinking pool of aerobically derived energy. While the correlation between MS and growth found in this study is not unique (Claireaux and Lefrancois, 2007; Jobling, 1981; Khan et al., 2014b), there is limited evidence linking key performance measures to MS under chronic temperature exposures (Clark et al., 2013). Other studies have found no association between temperature optimums for growth and MS in fish (Grans et al., 2014; Healy and Schulte, 2012; Norin et al., 2014) and it has become apparent that, although the OCLTT hypothesis

may be applicable in some species, it is not universal (Lefevre, 2016; Nilsson and Lefevre, 2016).

Although it was not quantified, egg deposition was evident during the growth trial at 15, 18 and 21°C but not at 24°C. This reproductive activity was unavoidable as wild *F. lapillum* spawn year round (Wellenreuther and Clements, 2007). Despite investment of energy in reproductive activity at 15–21°C, these fish still grew faster than fish at 24°C, where no reproductive activity was observed. The absence of reproductive activity at 24°C in this study could mean that spawning activity is constrained to cooler months if predicted warmer summertime conditions eventuate. Studies that specifically aim to address the influence of predicted future temperature regimes on the seasonality and performance of reproductive activity in this species would be of interest.

The pattern of SMR, $\dot{M}_{O_2,max}$ and MS in the present study contrasts with a previous work for *F. lapillum* where, despite increasing SMR with higher temperature, a peak in $\dot{M}_{O_2,max}$ at 24°C resulted in higher MS at 24°C than at lower temperatures (Khan et al., 2014a). However, Khan et al. (2014a) acclimated fish for only 4 weeks (versus 12 weeks in the present study) and fish may therefore have been in a different physiological state. The discrepancy in these findings highlights the importance of acclimation length in studies of this nature, and future studies investigating the time course of physiological adjustments in this species would be valuable. The different pattern of MS may also result from methodological differences, as $\dot{M}_{O_2,max}$ was estimated over a substantially longer period by Khan et al. (2014a), and this may have underestimated the $\dot{M}_{O_2,max}$ at lower temperatures.

The effect of warming on an annual cycle of growth

Few studies have assessed climate change with respect to seasonal growth. Despite the forecast of impaired growth at projected higher summer temperatures, when considered on an annual cycle and taking into account seasonal temperature fluctuations, the annual growth of *F. lapillum* might be slightly improved with a universal 1.5 and 3.0°C increase in temperature (Fig. 2). Warmer summer temperatures owing to climate change are expected to result in chronic exposure to temperatures beyond present-day conditions and compromised growth for *F. lapillum*. However, the influence of warming on growth during late autumn, winter and early spring may actually improve overall growth performance as temperature shifts closer to an optimal range (90% of maximum) (16.5–20.5°C; Fig. 1). The geographical range of this species extends to the southern end of New Zealand, where mean sea temperatures are cooler at all times of the year. Therefore, if southern populations have optimum temperature ranges for growth similar to the northern populations considered in this study, warming may serve to improve growth performance as temperatures are shifted closer to the optimum range for growth at all times of the year. The predicted growth rates, however, need to be interpreted with caution as they are based on average growth rates measured over an extended period (12 weeks) of continuous exposure to a single temperature. Wild populations face gradually rising and falling temperatures in spring and autumn and thus may be in a continual state of acclimation during these months, which could alter the temperature–growth relationship. It is also not known how gradual seasonal temperature changes between summer highs and winter lows, as occurs over 3 to 4 months in natural environments, impacts the temperature–growth relationship during the relatively thermally stable winter and summer periods that follow. While seasonal temperature fluctuations undoubtedly play a role in determining when a species can grow fast, other factors such as food availability and

competition also likely play an important role (Jones, 1986; Metcalfe, 1986). Consideration of how factors such as these are impacted by warming is required to develop a more complete picture of the growth response to climate change. Furthermore, the relationship between temperature and growth rate under laboratory conditions may not be applicable to wild populations.

The effect of warming on acute thermal tolerance

Chronically exposing *F. lapillum* to higher temperatures over 12 weeks increased its upper thermal tolerance limit (CT_{max}), and this has been reported for other fish species (Akhtar et al., 2013; Beitinger et al., 2000; Donelson, 2015; Drost et al., 2016; Healy and Schulte, 2012; McDonnell and Chapman, 2015; Sandblom et al., 2016; Zhang and Kieffer, 2014). However, thermal safety margins were reduced progressively because the increase in CT_{max} under acclimation to higher temperature was proportionately less than the increase in acclimation temperature (i.e. acclimation response ratios are less than 1). Low CT_{max} acclimation response ratios appear to be common among ectotherms and this suggests that plasticity in upper thermal tolerance has limited potential to reduce overheating risk (Gunderson and Stillman, 2015). Indeed, CT_{max} only increased 0.12°C between the acclimation temperatures of 21 and 24°C in the present study (Fig. 3), suggesting that *F. lapillum* exposed to temperatures beyond the present-day range do not have the acclimatory capacity to further increase CT_{max} . As a consequence, the thermal safety margin was reduced to its lowest value upon acclimation to 24°C.

While subtidal populations of *F. lapillum* reside in more thermally stable environments, this species also occupies intertidal rock pools where large temperature fluctuations occur. The significant reduction in thermal safety margin upon acclimation to a predicted future summertime temperature could therefore threaten survival or restrict available habitat for these intertidal populations. The one-off exposure to an acute temperature rise in the present study does not fully represent rock pool conditions and it is acknowledged that repeated acute temperature rises (as occurs over a tidal cycle) may further condition upper thermal tolerance. However, there was no evidence of repeated heat shock improving CT_{max} of the common killifish (*Fundulus heteroclitus*) (Healy and Schulte, 2012). Acute temperature rises in rock pools may also coincide with algal-mediated photosynthetic hyperoxia, but hyperoxia also appears to have no benefit to the CT_{max} of fish (Brijs et al., 2015; Healy and Schulte, 2012; Rutledge and Beitinger, 1989). Intergenerational or adaptive genetic responses in the upper thermal tolerance of *F. lapillum* could therefore be essential for mitigating overheating risk in a warmer future, although these too appear limited for the upper thermal tolerance of fish (Sandblom et al., 2016).

\dot{M}_{O_2} and acute thermal tolerance

The OCLTT hypothesis, with the differential availability of MS as the primary mechanism, seeks to unify explanations of thermal tolerance across the optimum to critical temperature range (Pörtner and Farrell, 2008). There is a difference, however, between how upper thermal tolerance is defined under the OCLTT hypothesis and the majority of studies investigating upper thermal tolerance in fish, which use CT_{max} (i.e. the temperature at which equilibrium is lost). Under the OCLTT hypothesis, the upper critical temperature (T_{crit}) is the point at which metabolic scope is nil (Farrell, 2016; Pörtner and Farrell, 2008), and how the CT_{max} fits within the OCLTT framework is unclear (Farrell, 2016; Healy and Schulte, 2012). Regardless, warm acclimation increases upper thermal tolerance in fish, and aerobic metabolism in fish with higher CT_{max} should reflect either an extension or retention of MS at high temperatures.

Theoretically, upper thermal tolerance could be increased through MS in two ways: (1) maximum aerobic capacity (i.e. $\dot{M}_{O_2,max}$) could be expanded at temperatures approaching critical limits, and/or (2) basal metabolic costs (i.e. SMR) could be reduced at temperatures approaching critical limits. In consideration of the first scenario, this study found no evidence of an expansion of maximum aerobic capacity with acclimation to warmer temperatures. In fact, despite having the highest CT_{max} , acclimation to the warmest temperature of 24°C restricted maximum aerobic capacity when measured at acclimation temperature (Fig. 1) and also limited maximum RMR at temperatures approaching critical limits (Fig. 4). Although $\dot{M}_{O_2,max}$ was not directly measured at high temperatures, the RMR approaching critical limits should reflect maximum capacity, because under the OCLTT framework SMR and $\dot{M}_{O_2,max}$ are the same at the upper critical temperature. This study finds more support for decreases in basal metabolic costs supporting improved upper thermal tolerance. Acclimation to increasingly warmer temperatures resulted in a lower RMR at equivalent temperatures during thermal ramping (Fig. 4A) and a lower RMR at 24 and 28°C was associated with higher CT_{max} (Fig. 5). These findings therefore fit the theory that physiological adjustments occur under warm acclimation to prevent the occurrence of high basal energetic costs (Norin et al., 2014; Pörtner, 2001; Sandblom et al., 2014; Seebacher et al., 2010). Lower metabolic rates in warm-acclimated fish may result from downregulation of mitochondrial function, which prevents excessively high metabolic rate during warming (Fangue et al., 2009; Pörtner, 2001) and could preserve MS at higher temperatures. However, despite the observed correlations in Fig. 5, it remains unclear whether lower RMR during thermal ramping actually plays a functional role in setting upper thermal tolerance because a clear difference in the metabolic rate of fish from the 21 and 24°C acclimation groups approaching their lethal thermal limit did not appear to dictate any difference in CT_{max} . Furthermore, experimental manipulations of oxygen transport capacity through anaemia (Brijs et al., 2015; Wang et al., 2014), hypoxia (Ern et al., 2016) and hyperoxia (Brijs et al., 2015) have little effect on upper thermal tolerance in fish.

Conclusions

In regard to chronic thermal tolerance, *F. lapillum* acclimated to 15, 18, 21 and 24°C (over 12 weeks) experience a decline in MS and growth fitness at 24°C. This finding is in support of the OCLTT hypothesis because of the correlation between metabolic and growth performance measures (Fig. 1). Unless genetic or transgenerational phenotypic acclimation can compensate, the main cause for concern would be that the metabolism and growth of *F. lapillum* would be sub-optimal with a 3°C temperature increase above present summertime SSTs. Few studies, however, assess climate change with respect to seasonal growth, and our modelling approach suggests that projected climate change will shift optimal growth to alternative times of the year and would thus result in an annual growth rate that is not impacted negatively (Fig. 2). Chronic warm acclimation only modestly increased upper thermal tolerance and, consequently, thermal safety margins declined at high acclimation temperatures (Fig. 3). In line with expectations under the OCLTT hypothesis, the observed increase in upper thermal tolerance under warm acclimation was associated with reduced RMR on acute thermal ramping (Fig. 4). In the context of global warming, the acute thermal tolerance of *F. lapillum* is a cause for concern because the ability of this species to withstand acute temperature change will decrease with every unit increase in peak chronic temperature. On that basis, the resilience of *F. lapillum* to future climate change will

be reduced progressively as temperatures increase and the distribution of this common coastal species may become limited to thermally stable sub-tidal habitats or higher latitudes to cope.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: T.J.M., N.A.H.; Methodology: T.J.M., N.A.H.; Validation: T.J.M.; Formal analysis: T.J.M.; Investigation: T.J.M.; Resources: T.J.M.; Writing - original draft: T.J.M.; Writing - review & editing: A.J.R.H., N.A.H.; Visualization: T.J.M.; Supervision: A.J.R.H., N.A.H.; Project administration: T.J.M., N.A.H.; Funding acquisition: A.J.R.H.

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