

Thermal windows and metabolic performance curves in a developing Antarctic fish

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Received: 26 May 2017 / Revised: 4 September 2017 / Accepted: 11 September 2017 / Published online: 7 October 2017
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Abstract For ectotherms, temperature modifies the rate of physiological function across a temperature tolerance window depending on thermal history, ontogeny, and evolutionary history. Some adult Antarctic fishes, with comparatively narrow thermal windows, exhibit thermal plasticity in standard metabolic rate; however, little is known about the shape or breadth of thermal performance curves of earlier life stages of Antarctic fishes. We tested the effects of acute warming (−1 to 8 °C) and temperature acclimation (2 weeks at −1, 2, 4 °C) on survival and standard metabolic rate in early embryos of the dragonfish *Gymnodraco acuticeps* from McMurdo Sound, Ross Island, Antarctica. Contrary to predictions, embryos acclimated to warmer temperatures did not experience greater mortality and nearly all embryos survived acute warming to 8 °C. Metabolic performance curve height and shape were both significantly altered after 2 weeks of development at −1 °C, with further increase in curve height, but not alteration of shape, with warm temperature acclimation. Overall metabolic rate temperature sensitivity (Q_{10}) from −1 to 8 °C varied from 2.6 to 3.6, with the greatest thermal sensitivity exhibited by embryos at earlier developmental stages. Interclutch variation in metabolic rates, mass, and development of simultaneously collected embryos was also documented. Taken together, metabolic performance curves provide insight into the costs of early development under warming temperatures, with the potential for thermal sensitivity to be modified by dragonfish phenology and magnitude of seasonal changes in temperature.

Keywords Temperature · Metabolism · Performance curves · Embryos · Polar fish · *Gymnodraco acuticeps*

Abbreviations

ANOVA	Analysis of variance
CTmax	Critical thermal maxima
GAMM	General additive mixed modeling
TPC	Thermal performance curve
SMR	Standard metabolic rate

Introduction

Thermal windows, the minimum and maximum temperatures that an organism can successfully tolerate provide general insight into the thermal niches of taxa (Fry 1971; Rezende et al. 2014) and have informed species distribution modeling, particularly in predicting response to climate change (Pearson and Dawson 2003; Sunday et al. 2012). Thermal performance curves (TPCs) measure a trait value or rate across a thermal gradient, allowing further assessment and integration of species' temperature sensitivity across thermal landscapes (Schulte et al. 2011; Huey et al. 2012; Magozzi and Calosi 2014). Incorporating different acclimation temperatures, time of exposure, and ontogeny allows more complex investigations of how organismal performance may be altered with warming associated with climate change (Hofmann and Todgham 2010; Komoroske et al. 2014; Sinclair et al. 2016).

The capacity to maintain oxygen supply to meet energetic demands under warming has been hypothesized to be an important determinant of how individuals, populations, and species cope with a changing environment (Pörtner and Knust 2007). Minimum oxygen consumption at rest (standard metabolic rate, SMR) provides a general proxy for

Communicated by G. Heldmaier.

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estimating baseline energetic requirements (Nelson 2016). Measurements of SMR across a thermal gradient allow for (a) assessing acute thermal limits where aerobic respiration is possible (thermal windows), (b) quantifying the role of temperature in shaping SMR (TPC), and (c) evaluating the effects of temperature acclimation on TPCs as all three contribute to predicting effects of altered thermal regimes (Verberk et al. 2015). As reviewed by Schulte (2015), initially, SMR is predicted to passively increase with acute temperature in an approximately exponential relationship commonly modeled by the Arrhenius equation (TPC shape). However, unlike in vitro biochemical reactions, at the whole organism level, the shape of the TPC can dampen as thermal limits are approached due to limits in the functional capacity (e.g., oxygen tissue delivery, mitochondrial bioenergetics, and maintenance of membrane-associated processes) to further increase and maintain SMR with rising temperature. For life stages (e.g., early embryos) where measurements of traditional “ecological death” indicators such as loss of equilibrium or cardiac arrhythmia are not possible, detecting flattening in metabolic TPC may provide a good alternate proxy of upper temperature tolerance before the onset of mortality (Terblanche et al. 2011). Thermal environment can further alter the height (value of metabolic rate at a given temperature) or shape of TPCs beyond passive kinetic effects through interactions with developmental processes (developmental plasticity) or physiological flexibility (thermal acclimation) to modify the costs of warming (Schulte et al. 2011; Sinclair et al. 2016). Ectotherms from stable thermal environments such as tropical and polar ecosystems are predicted to exhibit narrower thermal windows and thus greater sensitivity to current and future warming (Pörtner and Peck 2010; Somero 2012; although see Gunderson and Stillman 2015; Verberk et al. 2015).

Although critical for predicting species abundances, distributions, and responses to warming, relatively little work has compared thermal windows and thermal performance of early life stages and juvenile/adult fishes, particularly from stable thermal environments (Llopiz et al. 2014). In a systematic review, for temperate fish species, thermal window breadth (upper critical thermal limit – lower critical thermal limit) of early life stages is approximately half that of juvenile and adult fishes (11.6 vs. 20–25 °C; Rombough 1997). However, in tropical fish species where there is little seasonal variation in annual temperature, the few comparative studies suggest that upper thermal windows of embryos may lie within 2 °C of adults (Rombough 1997), with more recent indirect evidence from tropical damselfishes in the family Pomacentridae [reduced embryonic survival at 31 °C (Gagliano et al. 2007), negative growth, collapse of aerobic scope, and mortality in adults at 33–34 °C; (Rummer et al. 2014; Habary et al. 2017)]. Adult polar fish species exhibit similarly narrower thermal windows compared to

temperate fishes (Pörtner and Peck 2010; Somero 2010), but the thermal breadth of early life stages has not been well characterized.

Polar fishes are generally considered to be thermal specialists, with the ability to perform at sub-zero temperatures coming at the cost of lower thermal breadth and higher thermal sensitivity to increasing temperatures (Beers and Jayasundara 2015; Pörtner et al. 2017). In response to acute temperature increases, three species of adult Antarctic notothenioid fishes exhibited similar routine metabolic rate thermal performance curve shapes across a 4 °C increase above habitat temperature, while curve height inversely correlated with taxa habitat temperature (Sandersfeld et al. 2016). When faced with longer term warming, some species of adult polar fishes can partially or fully compensate to increased temperature (+5–6 °C) with acclimation through decreasing SMR (Robinson and Davidson 2008; Strobel et al. 2012; Drost et al. 2016b), while other species lack the ability to reduce the energetic costs of warming via SMR adjustment (Enzor et al. 2013; Egginton and Campbell 2016). While adults exhibit a varying amount of flexibility to cope with warming temperatures, lack of data investigating the response of early life stages of fishes to warmer temperatures has been identified as a limit to understanding the vulnerability of Antarctic fish species to impacts of climate change (Mintenbeck et al. 2012). With patterns of narrower thermal windows (Rombough 1997; Pörtner and Peck 2010), finite energetic resources and capabilities to buffer environmental change (Hamdoun and Epel 2007), and enhanced thermal sensitivity of cold-adapted physiological rate processes during development (Peck 2016), the early life stages are predicted to be more sensitive to temperature changes than adults. Recent studies suggest that temperatures 3–4 °C above ambient can profoundly alter hatching times and survival of embryos (Evans et al. 2005; Flynn et al. 2015) and larvae can acutely tolerate exposure to 9.5 °C above ambient temperature (Evans et al. 2012). How chronic exposure to warming limits and shapes acute thermal performance during early ontogeny has not been investigated.

To investigate the effects of warming on the temperature sensitivity and physiological performance in embryos of an Antarctic fish, we measured the effects of acute temperature change and short-term temperature acclimation on standard metabolic rate. The naked dragonfish, *Gymnodraco acuticeps*, is a benthic notothenioid that uses flat rocks to spawn its estimated 2500 benthic eggs that are guarded during the approximately 10 month period of development (Evans et al. 2005). From studies on several different taxa of adult Antarctic fishes, the upper limits of thermal windows fall in the range from 4 to 7 °C (Somero and Devries 1967; Windisch et al. 2014; Sandersfeld et al. 2015), and Ross Sea adult dragonfish acclimated to –1 °C can acutely tolerate temperatures of

13–14 °C during critical thermal maxima (CTMax) trials (Bilyk et al. 2012), comparable to other notothenioids from McMurdo Sound. Because of their long, slow development, dragonfish embryos experience the full range of seasonal temperature changes in the Ross Sea [from – 1.9 up to – 1 to 0 °C during brief summer warming (Cziko et al. 2014)], and at least some individuals can tolerate acclimation to warmer temperatures (1–2 °C) (Evans et al. 2005; Flynn et al. 2015). However, the ability to tolerate larger changes in environmental temperature, and the metabolic costs or adjustments required are unknown. To investigate these areas, we applied three approaches. To examine thermal tolerance, including comparisons with adult polar fish and non-polar embryos, we exposed dragonfish embryos to acute and chronic warming that we predicted that dragonfish embryos would be unable to tolerate (acute exposure up to 8 °C, chronic exposure to up to 4 °C). To test whether TPCs, and thus metabolic costs by acute temperature, are plastic during development, we created SMR curves for embryos at two different time points under three different temperatures regimes as we predicted that warmer temperatures could shift performance [reduce metabolic costs (height) or thermal sensitivity (shape)] via thermal acclimation in addition to potential ontogenetic changes. Finally, we quantified the standard metabolic rate of multiple clutches simultaneously collected from the environment to examine how subtle differences in developmental stage affected SMR.

Methods

Experiment 1: within clutch thermal performance

Collection and acclimation

Naked dragonfish (*Gymnodraco acuticeps*) embryos from a single clutch were collected on 25 November 2014, by SCUBA divers at the Jetty in McMurdo Sound, Ross Sea, Antarctica (77°51'14.04"S, 166°39'55.45"E). A single clutch was selected to ensure that developmental stage of the embryos was consistent at the start of the experiment. Short-term acclimation temperatures (“acclimation temperature”, – 1, 2, and 4 °C) were chosen based on a previous experiment where 2 °C was tolerated by dragonfish embryos, with some increase in mortality (Flynn et al. 2015), and 4 °C is commonly used in adult fish acclimation studies in the Ross Sea (Franklin et al. 2007; Enzor et al. 2013; Beers and Jayasundara 2015). Embryos were held in flow-through aquaria at – 1.5 to – 1.0 °C for 6 days, during which embryos were gently separated and viable individuals were randomly divided into groups of 33 in submerged mesh baskets and held at – 1 °C for at least 24 h prior to the start of the experiment. Each group of embryos was then assigned to one of three acclimation temperatures (– 1, 2, or 4 °C) with three replicate baskets per temperature (Fig. 1a). Temperature replicates were then introduced to their respective treatment, each replicate staggered by 1 day (i.e., one basket was placed at 2 °C on each of Day 1–3), and held for 14 days. For the two elevated temperature groups, embryos

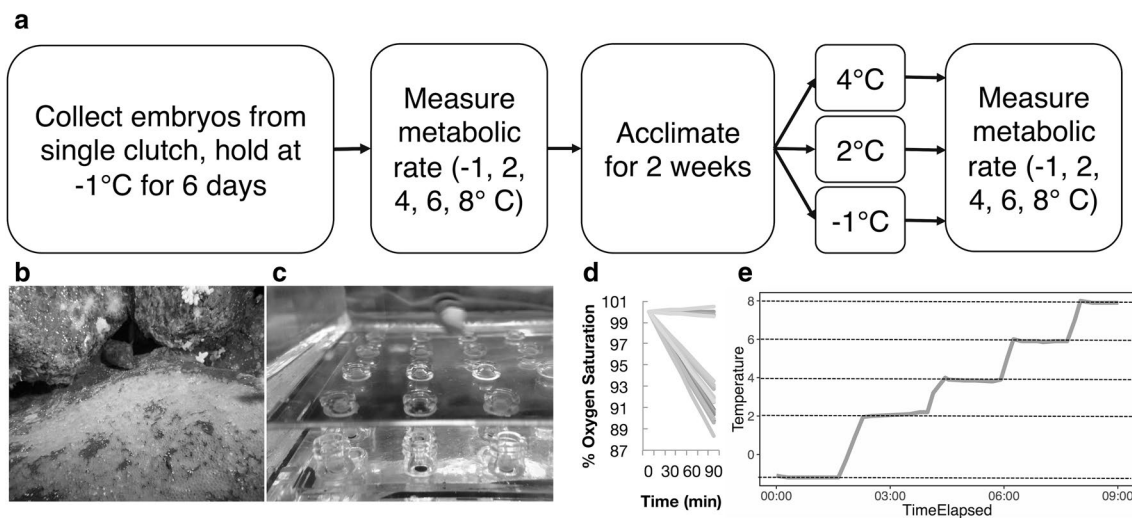


Fig. 1 Experiment 1 schematic. **a** Conceptual diagram and timeline of thermal acclimation and measures of thermal performance curves. **b** Adult dragonfish guarding a clutch of embryos at the study site (Photo R. Robbins). **c** Dragonfish embryos inside the open respirometry chambers. **d** Example of respirometry measurements at one temperature (oxygen saturation vs. time). Mean blank values (shown at

top of graph) are averaged and subtracted from individual embryo oxygen consumption rates. **e** Thermal performance was repeatedly measured across temperatures by alternating closed-chamber respirometry at set temperatures and ramping while replenishing oxygen at 0.1 °C/min

were held at 0 °C overnight and then ramped to 2 °C at 0.1 °C/min, with the 4 °C group additionally held at 2 °C for approximately 2 h before being ramped to 4 °C at the same rate. Another subset of embryos were measured on the same day that Day 1 embryos entered the acclimation temperatures (Time 0). This research was conducted in accordance with the US Federal animal welfare laws via approval and oversight by the University of California, Davis, Institutional Animal Care and Use Committee (protocol no. 18248) and in compliance with US regulations governing collection of Antarctic organisms.

Metrics

Survival, development, and morphometrics

After 14 days, survival was quantified in each replicate through visible inspection and non-viable embryos were removed from the experiment. Of the remaining embryos, per each replicate basket, 12 were reserved for whole organism metrics (6 for development staging and 6 for standard metabolic rate through measures of oxygen consumption). Live embryos were photographed under a stereoscope (Wild Heerbrugg scope with Canon Power Shot A630 camera and Carl Zeiss adaptor) in an environmental chamber at –1 °C along with a micrometer. After photography, embryos were briefly blotted dry and wet mass was measured to the nearest 0.01 mg, before being euthanized by snap freezing in liquid nitrogen. Development staging was assessed based on the previous studies of dragon fish embryos (Evans et al. 2005; Flynn et al. 2015). Specifically, embryos were categorized into one of four stages: lack of body axis visibility or differentiation (gastrulation), head and somites visible, but no pigment cells visible (early segmentation; Pigment 0), light pigment visible along notochord under magnification (segmentation; Pigment 1), and pigments easily visible by the naked eye, with a much broader coverage along notochord (segmentation; Pigment 2).

Standard metabolic rate performance curves

For acute temperature exposure, to construct the SMR TPCs, a series of temperatures were chosen (“acute temperature”, –1, 2, 4, 6, 8 °C) to balance including as many temperatures as possible within 1 day of measurement with breadth of temperature covered. The same acute temperatures have been used to measure cardiac output in adults (Franklin et al. 2007). Oxygen consumption rates of individual embryos were measured using a 24-well, 200 µl glass microplate pre-fitted with oxygen sensor spots (#CH10480, Loligo Systems, Tjele, Denmark) with each individual well (“vial”) sealed by a screwcap lid lined with a fluoropolymer. The microplate was then housed within an acrylic water bath connected to a

pump to maintain the desired temperature within the vials. Oxygen consumption was monitored in the dark using a microplate reader (#OX11900, Loligo Systems, Tjele, Denmark and SDR SensorDish Reader, PreSens, Regensburg, Germany) connected to a computer with PreSens software (SDR_v38). The plate was calibrated using 100 and 0% saturated water (air-bubbled and 1% sodium sulfite, respectively) at 2 °C.

To measure the effects of acute temperature change on metabolic performance, oxygen consumption of 18 embryos from each treatment was measured at –1, 2, 4, 6, and 8 °C acute temperatures using a repeated-measure design (Fig. 1b–e). Closed-chambered oxygen saturation was measured every 2 min, with total measurement time dependent on temperature to maintain O₂ saturation > 80% (6000s at –1 °C to 2000s at 8 °C). Between measurements, chambers were unsealed to refresh seawater (and restore 100% air saturation) in each vial as temperature was ramped to the next measurement value at 0.1 °C/min and embryos were visibly inspected for mortality. Six empty wells run concurrently were used to correct for background respiration. To calculate oxygen consumption rates per individual per temperature, slopes were calculated from O₂ saturation measurements using linear regression (average $r^2 = 0.98$). We removed the initial O₂ measurements to allow temperatures of sensor spots to stabilize within vials and to account for any disturbance stress associated with flushing vials before calculating slopes (first 3000s at –1 °C to 500 s at 8 °C). Rates were then transformed into nmol O₂ h^{–1} ind^{–1} using temperature, salinity, and atmospheric pressure (from McMurdo Station weather station) while accounting for embryo water displacement (using average wet mass of embryos photographed for development) and subtracting mean blank respiration.

Experiment 2: between clutch variation in metabolism

Collection and acclimation

SCUBA divers collected approximately 100 embryos from an additional 6 clutches on 2 December 2014, within the same general location at the Jetty in McMurdo Sound as Experiment 1 at depths between 15 and 20 m. Each clutch came from an independent rock and likely from six distinct females. Based on the total number of embryos/clutch and the physical attachment of embryos to each other, each clutch likely represented one female’s mature oocytes for the season (approximately 2500 embryos, Evans et al. 2005), although clutch paternity and the potential of multiple batch spawning by females are unknown. Dragonfish clutches were lab-acclimated at –1.5 to –1.0 °C for three days before experimentation, during which embryos were gently

separated from each other and non-viable embryos were removed.

Metrics

Oxygen consumption, development, and morphometrics

Oxygen consumption rate was measured in nine embryos/clutch at ambient temperature ($-1\text{ }^{\circ}\text{C}$) as described above across three separate trials in 1 day (three embryos/clutch/trial \times six clutches, with six additional blanks/trial). After each trial, each embryo was photographed for developmental staging and weighed (wet mass) as described in Experiment 1.

Data analysis

All analyses were completed in R (v3.3.0, R Core Team 2016) and all values are reported as mean \pm SEM unless otherwise stated. Model assumptions were checked visually and any violations in heterogeneity of variance were modeled as weighted variance following suggestions by Zuur et al. (2009). Differences in survival between acclimation temperatures were assessed using the number of live and dead embryos from each replicate ($n=3$) via logistic regression (generalized linear model, binomial family, “logit” link).

To quantify different aspects of the relationship between metabolic rate and temperature while providing the greatest ability to compare results with other studies, we analyzed the data using three complementary methods. First, to assess if there were differences in metabolic rates (A) within an acute temperature exposure between acclimation treatments (including Time 0) and (B) between temperature steps within an acclimation treatment, we performed two-way ANOVA with acclimation treatment and acute temperature as categorical fixed factors using the linear mixed effects function, `lme()` in the base “nlme” package. Due to the potential for non-linear aerobic performance across measurement temperatures and the repeated measurement design, general additive mixed modeling (“mgcv” package, Wood 2011) was chosen to assess differences in height and shape of the TPCs. Time and acclimation temperature were combined into one categorical variable with four levels (Time 0, -1 , 2 , $4\text{ }^{\circ}\text{C}$) with repeated measures per individual egg modeled as a random intercept effect with a correlated random slope by temperature step. To specifically test for differences in curve height and/or shape, the 2-week ambient temperature group was set as the baseline curve in ordered analysis. The other three groups were tested to see if they deviated in parametric estimates (curve height, described by the intercept value representing average metabolic rate across all temperatures) and the function of metabolic rate by acute temperature (curve shape, calculated by centering the mean to zero over

ramp temperatures, using restricted maximum likelihood for smoothness selection with $k=5$). Model assumptions were checked visually using `gam.check()`. Finally, as performance curves that follow Arrhenius relationships will linearize when the natural log is taken of the rate variable, we also present the data in this format.

Temperature sensitivity was also assessed by calculating the temperature coefficient, Q_{10} , with the following formula:

$$Q_{10} = \frac{R_2 \left(\frac{10}{T_2 - T_1} \right)}{R_1}$$

where R_1 and R_2 are oxygen consumption rates at temperatures T_1 and T_2 , respectively (Hochachka and Somero 2002). Routine metabolic rates, on average, approximately double for juvenile and adult fishes vs. triple for late-stage embryos and yolk-sac larvae for every $10\text{ }^{\circ}\text{C}$ increase in temperature (Rombough 1997). To capture the overall patterns in temperature sensitivity in early stage dragonfish embryos, we compared Q_{10} values calculated from metabolic rates between the lowest ($-1\text{ }^{\circ}\text{C}$) and highest temperatures ($8\text{ }^{\circ}\text{C}$) for each embryo by treatment group. Differences between treatment groups were assessed using a generalized least-squares model to account for heterogeneous variance between treatment groups, followed by Tukey’s post-hoc test after significant F values ($p < 0.05$) using the “nlme” and “lsmeans” packages. Q_{10} values were also calculated for each ramp step (i.e., from -1 to $2\text{ }^{\circ}\text{C}$) for each embryo but, due to a high amount of unexplained residual variance in the regression model, are presented only for visual comparison.

Results

Experiment 1: within clutch thermal performance

Acclimation temperature had no effect on survival ($\chi^2 = 0.47$, $df=1$, $p=0.49$), with overall high survival over the 2-week period ($-1\text{ }^{\circ}\text{C}$: $91.9 \pm 2.7\%$, $2\text{ }^{\circ}\text{C}$: $91.9 \pm 0.9\%$, and $4\text{ }^{\circ}\text{C}$: $88.9 \pm 2.7\%$, $n=3$). Mass was similar across all treatments ($21.6 \pm 0.1\text{ mg}$, $n=63$), while development stage reflected time and temperature acclimation. At the start of the experiment (Time 0), all embryos were at the gastrulation stage, whereas 2 weeks later at $-1\text{ }^{\circ}\text{C}$, the early eye formation was underway (Pigment 0); at $2\text{ }^{\circ}\text{C}$, pigments were visible along the notochord (Pigment 1), and at $4\text{ }^{\circ}\text{C}$, pigmentation was more prominent, extending to the head and yolk (Pigment 2).

During oxygen consumption trials, all but two embryos survived to the end and they were removed from further analysis along with three embryos with unusually high respiration at the start of the trial when inspected blinded

to treatment (final $n = 16–17$). Treatment (time by acclimation temperature) altered the relationship between acute temperature and metabolic rate. From the ANOVA analysis with planned group comparisons, there was a significant effect of acclimation temperature treatment, acute temperature, and their interaction on metabolic rate (acute temperature: $F_{4,252} = 346$, $p < 0.0001$; acclimation treatment: $F_{3,63} = 32$, $p < 0.0001$; acute temperature \times acclimation treatment: $F_{12,252}$; $p = 0.006$) (Fig. 2a). All metabolic rates significantly increased with increasing temperature except for the Time 0 group (held at -1 °C, but at an earlier stage of development) where the rate at 6 °C overlapped 4 and 8 °C. Within acute temperatures, Time 0 rates were always significantly lower than the other temperature acclimation treatments, 2 °C always overlapped either -1 or 4 °C, and -1 and 4 °C were significantly different at acute temperatures 2 and 4 °C.

From the general additive mixed modeling analysis, there were two significantly different TPC curve shapes with three different heights (Table 1). At Time 0, a linear relationship best modeled the increase in oxygen consumption with increasing temperature, whereas all temperature acclimation groups (-1 , 2, 4 °C) at 2 weeks exhibited non-linear changes in slope during the temperature ramp, notably between 4 and 6 °C (Fig. 2b). At -1 °C acclimation, over the course of 2 weeks of development, the height of the curve shifted as overall metabolic rate increased (3.9 ± 0.2 – 5.8 ± 0.2 nmol O₂ ind⁻¹ h⁻¹, $p < 0.0001$). At 2 and 4 °C acclimation, there was no additional difference in thermal performance curve shape ($p = 0.48$ – 0.57), and only the 4 °C, temperature acclimation group exhibited a significantly greater curve height (6.7 ± 0.3 nmol O₂ ind⁻¹ h⁻¹, $p = 0.013$). When the natural log of metabolic rate was plotted against temperature, Arrhenius relationships appear to be a reasonable approximation for the -1 , 2, and 4 °C temperature acclimation groups; however, the relationship fits poorly at Time 0 due to a break in the relationship between acute temperatures -1 and 2 °C (Fig. 2c).

Temperature sensitivity, when measured as the Q_{10} of metabolic rates across the entire temperature range of acute temperature exposures (-1 and 8 °C), was different among the temperature acclimation groups (Fig. 3, $F_{3,64} = 6.9$, $p = 0.0004$). Q_{10} was significantly greater at Time 0 (3.6 ± 0.3) compared to the 2-week groups at all acclimation temperatures (-1 °C: 2.9 ± 0.2 ; 2 and 4 °C: 2.6 ± 0.1). When Q_{10} was qualitatively examined between acute temperature intervals during MO₂ trials (Fig. 3), the Time 0 group exhibited the greatest thermally sensitivity (deviation from overall Q_{10}) between -1 and 2 °C, whereas the temperature acclimated embryos exhibited greater thermal sensitivity at the warmest temperature intervals (4–6, 6–8 °C).

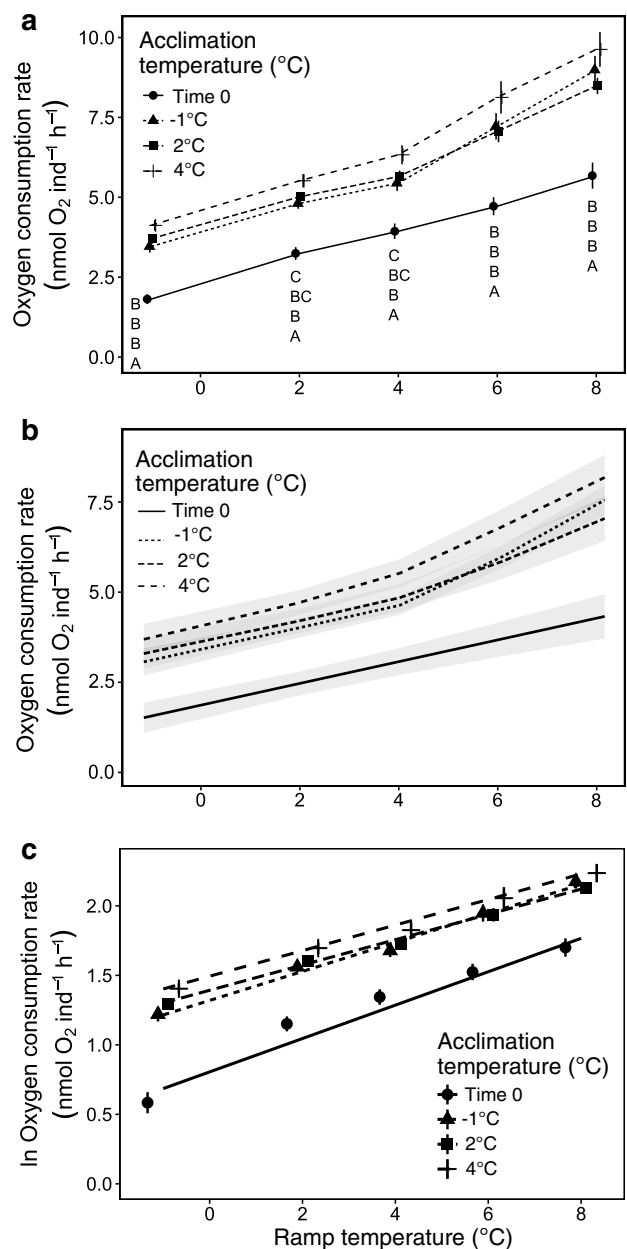


Fig. 2 Effects of acute (-1 , 2, 4, 6, 8 °C), and acclimation temperature treatment (Time 0, -1 , 2, 4 °C) on metabolic rate of dragonfish embryos ($n = 16–18$). Time 0 embryos were at gastrulation stage and held at -1 °C, and -1 , 2, 4 °C embryos (14 days later) were undergoing segmentation. Letters are in order, starting from the bottom, by Time 0, -1 , 2, 4 °C. **a** Mean oxygen consumption rate (\pm SEM), with non-shared letters representing significantly different least-squared means of treatment within an acute temperature value, **b** Thermal performance curves (\pm SEM), generated from generalized additive mixed modeling, and **c** thermal performance curves with the natural log of metabolic rate, with a fitted linear model

Experiment 2: between clutch variation in metabolism

The six clutches collected differed in developmental stage, likely reflecting differences in lay date (unknown),

Table 1 Comparisons of generalized additive mixed models of metabolic rate as a function of temperature, $f(T)$, referenced to the curve from $-1\text{ }^\circ\text{C}$ acclimation

Fixed effects	Parametric				Smooth		
	Intercept estimate	SE	T	p value	e.d.f.	F	p value
$f(T)$ at 2 w, $-1\text{ }^\circ\text{C}$ (ambient)	5.98	0.21	28.24	<0.0001	3.563	65.14	<0.0001
Deviation of $f(T)$ for time 0, $-1\text{ }^\circ\text{C}$ (ambient)	-2.10	0.30	-7.03	<0.0001	2.608	7.76	<0.0001
Deviation of $f(T)$ for 2 w, $2\text{ }^\circ\text{C}$ (+3 $^\circ\text{C}$)	0.00	0.30	0.01	0.955	1.002	1.69	0.195
Deviation of $f(T)$ for 2 w, $4\text{ }^\circ\text{C}$ (+5 $^\circ\text{C}$)	0.76	0.30	2.50	0.013	1.001	0.02	0.894

Parametric effects test the significance of treatment on the “height” of the thermal performance curve (TPC) (intercept estimate, standard error (SE), T value (T), and p value), while the smooth tests the significance of treatment on the “shape” of the TPC (estimated degrees freedom (e.d.f.), F value (F), and p value). The intercept estimate is the overall mean metabolic rate for each treatment

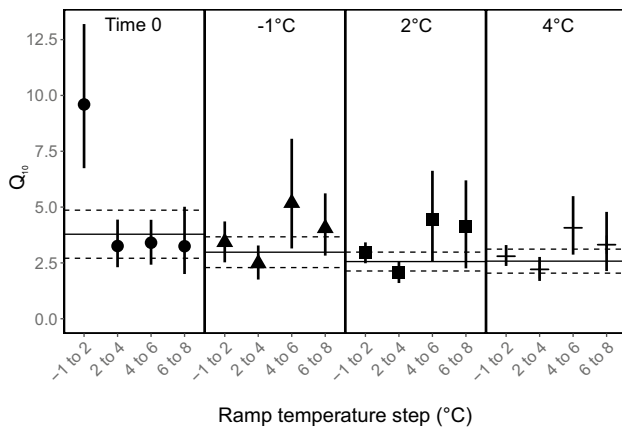


Fig. 3 Effects of acute temperature ($-1, 2, 4, 6, 8\text{ }^\circ\text{C}$) increase on thermal sensitivity (Q_{10}) of metabolic rate of dragonfish embryos ($n=16-18$) by acclimation temperature treatment (Time 0, $-1, 2, 4\text{ }^\circ\text{C}$). Time 0 embryos were at gastrulation stage and held at $-1\text{ }^\circ\text{C}$, and $-1, 2, 4\text{ }^\circ\text{C}$ embryos (14 days later) were undergoing segmentation. Lines represent overall Q_{10} between -1 and $8\text{ }^\circ\text{C}$ (mean ± 1 SD), while individual points are step-wise Q_{10} values (mean \pm SEM).

but largely overlapped with the developmental stages of embryos used in Experiment 1 (Table 2). The earliest embryos had visible body axis formation but lack of clear head differentiation (Pre-segmentation: C2, 3, and 4), there was a slightly more developed group with visible early eyes and segmentation [segmentation (Pigment 0) C5 and 6], and one clutch had a majority of individuals with pigments developing along the notochord [segmentation (Pigment 1) C1]. Metabolic rate ranged from 1.2 ± 0.2 to $2.7 \pm 0.2\text{ nmol O}_2\text{ ind}^{-1}\text{ h}^{-1}$ and wet mass ranged from 14.7 ± 0.3 to $21.6 \pm 0.2\text{ mg}$. Results are presented with data from Experiment 1 (Table 2) ordered by development from earliest to latest.

Discussion

Investigating ontogenetic shifts in TPCs has been identified as an understudied area of research in predicting species response to climate change (Sinclair et al. 2016). This study

Table 2 Summary of dragonfish embryo trait values from one clutch from the thermal performance experiment (TPC, Experiment 1) and six independent clutches (Clutches, C1–6, Experiment 2) collected on 2 December 2014, from McMurdo Station, and measured on 5 December 2014

Majority development stage	Experiment	Group	n_{MO_2}	MO_2 at $-1\text{ }^\circ\text{C}$ (nmol $\text{ind}^{-1}\text{ h}^{-1}$)	n_{mass}	Wet mass (mg)
Gastrulation	Clutches	C3	9	1.2 ± 0.2	9	14.7 ± 0.3
Gastrulation	Clutches	C2	9	1.6 ± 0.1	9	18.0 ± 0.4
Gastrulation	TPC	Time 0	17	1.8 ± 0.2	9	22.2 ± 0.3
Gastrulation	Clutches	C4	9	2.4 ± 0.2	9	19.6 ± 0.2
Early segmentation (Pigment 0)	Clutches	C5	9	2.5 ± 0.1	9	21.6 ± 0.2
Early segmentation (Pigment 0)	Clutches	C6	9	2.8 ± 0.2	9	18.2 ± 0.1
Early segmentation (Pigment 0)	TPC	$-1\text{ }^\circ\text{C}$	17	3.5 ± 0.2	18	21.5 ± 0.2
Segmentation (Pigment 1)	Clutches	C1	9	2.7 ± 0.2	9	20.2 ± 0.3
Segmentation (Pigment 1)	TPC	$+2\text{ }^\circ\text{C}$	17	3.7 ± 0.2	18	21.7 ± 0.1
Segmentation (Pigment 2)	TPC	$+4\text{ }^\circ\text{C}$	16	4.1 ± 0.2	18	21.3 ± 0.1

Values are means \pm SEM

provides the first individual-level measurements of TPCs in Antarctic fish embryos by measuring standard metabolic rate across five temperatures at different early developmental stages. Contrary to expectations, we found no evidence that the majority of dragonfish embryos exceeded their thermal tolerance limits during acute exposure up to 8 °C and following short-term acclimation to 2 and 4 °C. While there were significant differences in the height and shape of the TPCs after 2 weeks of additional development at –1 °C, embryos held at warmer acclimation temperatures experienced no (2 °C) to little (4 °C) additional increases in curve height (increase in SMR at a given temperature). Overall metabolic rate temperature sensitivity (Q_{10}) across a range of temperatures ($\Delta 9$ °C, –1 to 8 °C) varied from 2.6 to 3.6, with the greatest thermal sensitivity exhibited by Time 0 embryos at the earliest developmental stage. Additional metabolic rate measurements of individuals from six separate clutches taken at environmental temperatures (–1 °C) provide an estimate of intrapopulation variability in standard metabolic rate along with variation in development timing and maternal provisioning.

We did not detect upper thermal limits for survival in dragonfish embryos during short-term (2 weeks) temperature acclimation to 2 and 4 °C. While this period represents a relatively small fraction (14 days) of total development time [approximately 10 months at environmental temperatures (Evans et al. 2005)] at one particular stage (transition from gastrulation to segmentation), these data fill an important data gap examining the thermal windows of Antarctic fish embryos. In contrast to a previous study from our group finding enhanced mortality at 2 °C acclimation temperature (Flynn et al. 2015), the current study started with embryos at a later developmental stage, and therefore, it may be that dragonfish are more negatively affected by increased temperature earlier in development, similar to other cold-adapted fish embryos (Rombough 1997; Cingi et al. 2010). It is also possible that most mortality occurs early in development (Dahlke et al. 2016) or that exposure to elevated temperatures can have negative carry-over effects that impact hatching or larval fitness (Hamdoun and Epel 2007; Drost et al. 2016a). Within that context, our results suggest that the thermal window of dragonfish embryos may include or exceed 4 °C during periods of early ontogeny. Arctic cod (*Boreogadus saida*) embryos develop at the same near-freezing temperature as the Antarctic dragonfishes, yet they successfully hatched and survived to reproduction when reared at 3.5 °C (Drost et al. 2016a). While the upper thermal window of adult dragonfish has yet to be characterized, other Antarctic fishes from the Ross Sea exhibited 33% mortality during a month acclimation at 2–4 °C (Sandersfeld et al. 2015) or 50% mortality during a week at >5–6 °C (Somero and Devries 1967), although others have successfully held adults for weeks to months at 4–6 °C without mortalities (Franklin

et al. 2007; Robinson and Davison 2008; Enzor et al. 2013; Beers and Jayasundara 2015; Egginton and Campbell 2016). In their natural environment, however, eight larval Antarctic fish species from the lower latitude peninsula region were all found in –0.8 to 1.3 °C water temperatures (La Mesa et al. 2016), and thus, the early life stages may prefer cooler water or be constrained to cooler thermal windows due to other abiotic or biotic forces.

Acute thermal tolerance limits reflect the point of collapse of organismal function. Our finding that dragonfish embryos could maintain metabolic function at increasing temperatures up to 8 °C (+ $\Delta 9$ °C) over the course of a day exceeds the previous warmest acute temperature exposure of Antarctica fish embryos [6 °C for several hours, near-hatching stage of the Antarctic silverfish *Pleuragramma antarcticum* (Evans et al. 2012)]. Our minimum estimate of upper acute thermal tolerance is equal to or within other fish embryo measurements as the tropical zebrafish (28.5 °C) post-blastula embryos can tolerate an acute 1 h exposure to + $\Delta 8.5$ °C (but not + $\Delta 11.5$ °C) (Krone et al. 1997) and the cold-adapted lake whitefish (*Coregonus clupeaformis*) (2 °C) at fin-flutter stage can withstand at least 4 h at + $\Delta 9$ °C (Stefanovic et al. 2016). As newly hatched larvae of one Antarctic fish species have similar CT_{max} values (14.6 °C) (Evans et al. 2012) as a range of adult fishes (13–18 °C) (Bilyk and Devries 2011; Beers and Sidell 2011), maximum acute thermal tolerance may be more ontogenetically conserved in stably cold environments as in warm tropic environments (Rombough 1997; Ospina and Mora 2004). Interestingly, Antarctic sea urchin embryos (*Sterechinus neumayeri*) can survive 1 h of acute exposure to 15–20 °C (Kapsenberg and Hofmann 2014), but normal development ceases under chronic elevated temperature above 3 °C (Karelitz et al. 2016).

Thermal performance curves of standard metabolic rate altered during early development, but very little with short-term temperature acclimation. All treatment groups increased metabolic rate as acute temperature increased, with no indication of exceeding a TPC optima below the highest temperature tested. As expected, more developed embryos exhibited greater metabolic rates (measured per embryo), although warmer acclimation temperatures with enhanced development only modestly increased SMR at the highest acclimation temperature (4 °C). In zebrafish, the increase in basal metabolic rate between gastrulation and segmentation is due to an increase in mitochondrial respiration (specifically proton leak), suggesting enhanced mitochondrial mass and function during segmentation (Huang et al. 2013). As we did not specifically test the metabolic effects of temperature acclimation at the exact same stage during segmentation, it is possible that basal metabolic costs are broadly similar during this period. Alternatively, warmer temperature acclimation might plastically reduce

SMR. Developing in warmer conditions can lead to lower adult SMR TPC height in fishes (Schaefer and Walters 2010) much like reversible thermal acclimation in adults (Seebacher et al. 2014; Drost et al. 2016b), but generally, embryos are thought to have limited capacity to metabolically buffer increased temperature effects (Rombough 1997). However, switching embryos to warmer developmental temperatures after gastrulation through organogenesis in the cold stenothermic lake whitefish resulted in lower mass-specific metabolic rates compared to constant warm-acclimated individuals (Eme et al. 2015). Understanding the mechanistic role of metabolism in temperature and developmental plasticity, along with contributing variables such as maternal effects (Giesing et al. 2011) and other environmental variables (Barrionuevo and Burggren 1999; Rudneva 2013; Flynn et al. 2015), may provide more insight into the persistence of developmental temperature-dependent phenotypes into adulthood (Scott and Johnston 2012; Hurst et al. 2012; Bizuayehu et al. 2015).

Thermal sensitivity, as calculated by Q_{10} values, provides a complementary method of comparing thermal performance of embryos across a thermal gradient. We found that embryos earlier in development (gastrulation) exhibited greater acute thermal sensitivity ($Q_{10} > 3$), suggesting a greater increase in metabolic cost per temperature increase. This stage also experienced the greatest step-wise increase in sensitivity between acute temperatures of -1 and 2 °C, with reduced, consistent temperature sensitivity across the remaining acute temperature increase steps. In comparison, embryos measured during somitogenesis (i.e., following 2 weeks of development) were less thermally sensitive overall (Q_{10} values between 2 and 3); however, embryos at this later stage exhibited an increase in magnitude and variability of step-rate Q_{10} values at warmer acute temperature increases (from 4 to 6 and 6 to 8 °C). As oxygen consumption reflects the capacity to deliver oxygen to mitochondria, efficiency of aerobic ATP generation, and energy demands, the difference in thermal sensitivity between embryonic stages with increasing temperature may reflect changes in any of these processes. One possibility is that as more basal metabolism is devoted to the non-ATP generating mitochondrial protein leak during zebrafish embryo development (Huang et al. 2013), rising temperatures may exceed mitochondrial coupling efficiency optima of embryos (Dahlke et al. 2016). This inefficiency would lead to greater oxygen demand per temperature increase, along with any other increases in basal metabolic demands (e.g., cellular stress response or protein turnover). Comparatively, we found SMR thermal sensitivity in dragonfish embryos to be broadly similar to other early stages of fishes ($Q_{10} = 2-3$; Rombough 1997), newly hatched Antarctic fish larvae ($Q_{10} = 2.45$; Evans et al. 2012), and within the range of values reported for Antarctic adult fishes ($Q_{10} = 1.6-4.5$;

Franklin et al. 2007; Jayasundara et al. 2013; Egginton and Campbell 2016).

Within the local dragonfish population, we provide the first documentation of trait variation in embryos from different clutches. SMR at ambient temperature generally increased with development in embryos collected at the same time from six different clutches (Table 2). While the previous studies on individual and pooled dragonfish embryos during similar stages were unable to detect developmental increases in rates of oxygen consumption at -1 °C (Evans et al. 2006; Flynn et al. 2015), the higher throughput methods used in this study at the individual embryo level provide enough increased sensitivity to capture differences. Clutches also exhibited intra- and intergroup variation in phenotype (whole egg wet mass) and phenology (development stage by calendar date). As results from this study demonstrate that the relative metabolic cost of acute warming depends on development stage, the small seasonal increase in summer temperature ($+\Delta 0.5$ to 1.7 °C increase above -1.8 °C, Cziko et al. 2014) may differentially alter fitness depending on reproductive timing of spawning (October–November). In addition, egg size correlates with larval fitness in many other fish taxa (Pepin et al. 1997; Einum and Fleming 2002), likely reflecting maternal size and fecundity as in another Antarctic fish genus, *Lepidonotothen*, with a similar reproductive strategy (nest-guarding of demersal eggs; La Mesa et al. 2017). Standing variation in embryo phenotype, whether resulting from potential genotypic, epigenetic, or environmental origins (Pakkasmaa et al. 2006; Donelson et al. 2011; Whitney et al. 2013), suggests the possibility for selection to further tune phenotypes under future warming, sea ice changes, altered carbonate chemistry, and shifts in productivity timing that the Ross Sea is predicted to experience under global climate change (Smith et al. 2012; Kapsenberg et al. 2015; Petrou et al. 2016).

Using thermal performance curves and aerobic metabolism to predict how species will respond to future warming has a rich history in physiological ecology (Fry 1971; Rombough 1988; Pörtner and Knust 2007), and recent reviews have highlighted the complexities, cautions, areas of limitations, and exciting questions that still need to be addressed (Schulte et al. 2011; Rezende et al. 2014; Schulte 2015; Verberk et al. 2015; Sinclair et al. 2016). While this study provides the first thermal performance curves for Antarctic fish embryos, and one of the few studies to measure metabolic rate at the individual embryo, we were only able to capture a short portion of the effects of temperature acclimation and acute temperature exposure on the development of dragonfish embryos. Chronic acclimation to warmer temperatures may lead to more severe effects or allow time for negative effects (such as developmental abnormalities) to manifest. Dragonfish embryos may become more thermally sensitive at later stages of development like some fish taxa, although

this pattern is less common (Rombough 1997). Differing methods for measuring upper thermal windows can result in different estimates, although studies of adult Antarctic fishes with different ramping rates have found relatively similar (within about 1 °C) estimates (Beers and Jayasundara 2015). Even within the small population of dragonfish at the Jetty intake in front of McMurdo Station, there is some variation in development timing and metabolic demand by clutch, suggesting that there may be differences in response to warming. Future research elucidating thermal windows in the early life stages of Antarctic fishes will provide further insight into whether adults, larvae, and embryos share thermal tolerances, and if taxa-specific and location-specific differences measured in adults exist at earlier ontogenetic stages.

Acknowledgements We thank the United States Antarctic Program and Lockheed Martin for logistical and field support at McMurdo Station, Antarctica, including SCUBA divers Rob Robbins and Steve Rupp. Brittany Davis and Dr. Nathan Miller provided invaluable experimental assistance, and Prof. Nann Fangue contributed helpful discussion and feedback to the writing of this paper. This work was supported by the National Science Foundation (NSF ANT-1142122) to A.E.T.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Barrionuevo W, Burggren W (1999) O₂ consumption and heart rate in developing zebrafish (*Danio rerio*): influence of temperature and ambient O₂. *Am J Physiol Regul Integr Comp Physiol* 276:R505–R513
- Beers JM, Jayasundara N (2015) Antarctic notothenioid fish: what are the future consequences of ‘losses’ and ‘gains’ acquired during long-term evolution at cold and stable temperatures? *J Exp Biol* 218:1834–1845. doi:10.1242/jeb.116129
- Beers JM, Sidell BD (2011) Thermal tolerance of Antarctic notothenioid fishes correlates with levels of circulating hemoglobin. *Physiol Biochem Zool* 84:353–362. doi:10.1086/660191
- Bilyk KT, Devries AL (2011) Heat tolerance and its plasticity in Antarctic fishes. *Comp Biochem Phys A* 158:382–390. doi:10.1016/j.cbpa.2010.12.010
- Bilyk KT, Evans CW, Devries AL (2012) Heat hardening in Antarctic notothenioid fishes. *Polar Biol* 35:1447–1451
- Bizuayehu TT, Johansen SD, Puvanendran V et al (2015) Temperature during early development has long-term effects on microRNA expression in Atlantic cod. *BMC Genomics* 16:305. doi:10.1186/s12864-015-1503-7
- Cingi S, Keinänen M, Vuorinen PJ (2010) Elevated water temperature impairs fertilization and embryonic development of whitefish *Coregonus lavaretus*. *J Fish Biol* 76:502–521. doi:10.1111/j.1095-8649.2009.02502.x
- Cziko PA, Devries AL, Evans CW, Cheng C-HC (2014) Antifreeze protein-induced superheating of ice inside Antarctic notothenioid fishes inhibits melting during summer warming. *Proc Natl Acad Sci USA* 111:14583–14588. doi:10.1073/pnas.1410256111
- Dahlke FT, Leo E, Mark FC et al (2016) Effects of ocean acidification increase embryonic sensitivity to thermal extremes in Atlantic cod, *Gadus morhua*. *Glob Change Biol* 23:1499–1510. doi:10.1111/gcb.13527
- Donelson JM, Munday PL, McCormick MI, Pitcher CR (2011) Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nat Clim Change* 2:30–32. doi:10.1038/nclimate1323
- Drost HE, Fisher J, Randall F et al (2016a) Upper thermal limits of the hearts of Arctic cod *Boreogadus saida*: adults compared with larvae. *J Fish Biol* 88:718–726. doi:10.1111/jfb.12807
- Drost HE, Lo M, Carmack EC, Farrell AP (2016b) Acclimation potential of Arctic cod (*Boreogadus saida*) from the rapidly warming Arctic Ocean. *J Exp Biol* 219:3114–3125. doi:10.1242/jeb.140194
- Egginton S, Campbell HA (2016) Cardiorespiratory responses in an Antarctic fish suggest limited capacity for thermal acclimation. *J Exp Biol* 219:1283–1286. doi:10.1242/jeb.130963
- Einum S, Fleming IA (2002) Does within-population variation in fish egg size reflect maternal influences on optimal values? *Am Nat* 160:756–765. doi:10.1086/343876
- Eme J, Mueller CA, Manzon RG et al (2015) Critical windows in embryonic development: shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (*Coregonus clupeaformis*) embryos and hatchlings. *Comp Biochem Physiol A Mol Integr Physiol* 179:71–80. doi:10.1016/j.cbpa.2014.09.005
- Enzor LA, Zippay ML, Place SP (2013) High latitude fish in a high CO₂ world: synergistic effects of elevated temperature and carbon dioxide on the metabolic rates of Antarctic notothenioids. *Comp Biochem Phys A* 164:154–161. doi:10.1016/j.cbpa.2012.07.016
- Evans CW, Cziko P, Cheng C-HC, Devries AL (2005) Spawning behaviour and early development in the naked dragonfish *Gymnodraco acuticeps*. *Antarct Sci* 17:319–327. doi:10.1017/S0954102005002749
- Evans CW, Pace L, Cziko PA et al (2006) Metabolic energy utilization during development of Antarctic naked dragonfish (*Gymnodraco acuticeps*). *Polar Biol* 29:519–525
- Evans CW, Williams DE, Vacchi M et al (2012) Metabolic and behavioural adaptations during early development of the Antarctic silverfish, *Pleuragramma antarcticum*. *Polar Biol* 35:891–898. doi:10.1007/s00300-011-1134-7
- Flynn EE, Bjelde BE, Miller NA, Todgham AE (2015) Ocean acidification exerts negative effects during warming conditions in a developing Antarctic fish. *Conserv Physiol* 3:cov033. doi:10.1093/conphys/cov033
- Franklin CE, Davison W, Seebacher F (2007) Antarctic fish can compensate for rising temperatures: thermal acclimation of cardiac performance in *Pagothenia borchgrevinki*. *J Exp Biol* 210:3068–3074. doi:10.1242/jeb.003137
- Fry FEJ (1971) The effect of environmental factors on the physiology of fish. *Fish Phys* 6:1–98. doi:10.1016/S1546-5098(08)60146-6
- Gagliano M, McCormick MI, Meekan MG (2007) Temperature-induced shifts in selective pressure at a critical developmental transition. *Oecologia* 152:219–225. doi:10.1007/s00442-006-0647-1
- Giesing ER, Suski CD, Warner RE, Bell AM (2011) Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. *Proc R Soc B* 278:1753–1759. doi:10.1146/annurev.neuro.24.1.1161
- Gunderson AR, Stillman JH (2015) Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc R Soc B* 282:20150401. doi:10.1098/rspb.2015.0401
- Habary A, Johansen JL, Nay TJ et al (2017) Adapt, move or die—how will tropical coral reef fishes cope with ocean warming? *Glob Change Biol* 23:566–577. doi:10.1111/gcb.13488
- Hamdoun A, Epel D (2007) Embryo stability and vulnerability in an always changing world. *Proc Natl Acad Sci USA* 104:1745–1750. doi:10.1073/pnas.0610108104

- Hochachka PW, Somero GN (2002) Biochemical adaptation: mechanism and process in physiological evolution. Oxford University Press, New York
- Hofmann GE, Todgham AE (2010) Living in the now: physiological mechanisms to tolerate a rapidly changing environment. *Annu Rev Physiol* 72:127–145. doi:10.1146/annurev-physiol-021909-135900
- Huang S-H, Huang K-S, Yu C-H, Gong H-Y (2013) Metabolic profile analysis of a single developing zebrafish embryo via monitoring of oxygen consumption rates within a microfluidic device. *Biomicrofluidics* 7:064107. doi:10.1063/1.4833256
- Huey RB, Kearney MR, Krockenberger A et al (2012) Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Phil Trans R Soc B* 367:1665–1679. doi:10.1098/rstb.2012.0005
- Hurst TP, Munch SB, Lavelle KA (2012) Thermal reaction norms for growth vary among cohorts of Pacific cod (*Gadus macrocephalus*). *Mar Biol* 159:2173–2183. doi:10.1007/s00227-012-2003-9
- Jayasundara N, Healy TM, Somero GN (2013) Effects of temperature acclimation on cardiorespiratory performance of the Antarctic notothenioid *Trematomus bernacchii*. *Polar Biol* 36:1047–1057. doi:10.1007/s00300-013-1327-3
- Kapsenberg L, Hofmann GE (2014) Signals of resilience to ocean change: high thermal tolerance of early stage Antarctic sea urchins (*Sterechinus neumayeri*) reared under present-day and future pCO₂ and temperature. *Polar Biol* 37:967–980. doi:10.1007/s00300-014-1494-x
- Kapsenberg L, Kelley AL, Shaw EC et al (2015) Near-shore Antarctic pH variability has implications for the design of ocean acidification experiments. *Sci Rep* 5:9638. doi:10.1038/srep09638
- Karelitz SE, Uthicke S, Foo SA et al (2016) Ocean acidification has little effect on developmental thermal windows of echinoderms from Antarctica to the tropics. *Glob Change Biol* 23:657–672. doi:10.1111/gcb.13452
- Komoroske LM, Connon RE, Lindberg J et al (2014) Ontogeny influences sensitivity to climate change stressors in an endangered fish. *Conserv Physiol* 2:cou008. doi:10.1093/conphys/cou008
- Krone PH, Lele Z, Sass JB (1997) Heat shock genes and the heat shock response in zebrafish embryos. *Biochem Cell Biol* 75:487–497
- La Mesa M, La Mesa G, Catalano B, Jones CD (2016) Spatial distribution pattern and physical–biological interactions in the larval notothenioid fish assemblages from the Bransfield Strait and adjacent waters. *Fish Oceanogr* 25:624–636. doi:10.1111/fog.12178
- La Mesa M, Riginella E, Catalano B et al (2017) Maternal contribution to spawning and early life-history strategies of the genus *Lepidonotothen* (Nototheniidae, Perciformes) along the southern Scotia Arc. *Polar Biol*. doi:10.1007/s00300-016-2068-x
- Llopiz JK, Cowen RK, Hauff MJ et al (2014) Early life history and fisheries oceanography: new questions in a changing world. *Oceanography* 27:26–41
- Magozzi S, Calosi P (2014) Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Glob Change Biol* 21:181–194. doi:10.1111/gcb.12695
- Mintenbeck K, Barrera-Oro E, Brey T et al (2012) Impact of climate change on fishes in complex Antarctic ecosystems. *Adv Ecol Res* 46:351–426
- Nelson JA (2016) Oxygen consumption rate v. rate of energy utilization of fishes: a comparison and brief history of the two measurements. *J Fish Biol* 88:10–25. doi:10.1111/jfb.12824
- Ospina AF, Mora C (2004) Effect of body size on reef fish tolerance to extreme low and high temperatures. *Environ Biol Fish* 70:339–343. doi:10.1023/B:EBFL.0000035429.39129.34
- Pakkasmaa S, Penttinen O-P, Piironen J (2006) Metabolic rate of Arctic charr eggs depends on their parentage. *J Comp Physiol B* 176:387–391. doi:10.1007/s00360-005-0057-4
- Pearson RG, Dawson TP (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Glob Ecol Biogeogr* 12:361–371
- Peck LS (2016) A cold limit to adaptation in the sea. *Trends Ecol Evol* 31:13–26. doi:10.1016/j.tree.2015.09.014
- Pepin P, Orr DC, Anderson JT (1997) Time to hatch and larval size in relation to temperature and egg size in Atlantic cod (*Gadus morhua*). *Can J Fish Aquat Sci* 54:2–10. doi:10.1139/f96-154
- Petrou K, Kranz SA, Trimborn S et al (2016) Southern Ocean phytoplankton physiology in a changing climate. *J Plant Physiol* 203:135–150. doi:10.1016/j.jplph.2016.05.004
- Pörtner H, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315:95–97
- Pörtner HO, Peck MA (2010) Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *J Fish Biol* 77:1745–1779. doi:10.1111/j.1095-8649.2010.02783.x
- Pörtner HO, Bock C, Mark FC (2017) Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. *J Exp Biol* 220:2685–2696. doi:10.1007/s00227-012-2073-8
- Rezende EL, Castañeda LE, Santos M (2014) Tolerance landscapes in thermal ecology. *Funct Ecol* 28:799–809. doi:10.1111/1365-2435.12268
- Robinson E, Davison W (2008) Antarctic fish can survive prolonged exposure to elevated temperatures. *J Fish Biol* 73:1676–1689. doi:10.1111/j.1095-8649.2008.02041.x
- Rombough PJ (1988) Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. *Can J Zool* 66:651–660. doi:10.1139/z88-097
- Rombough PJ (1997) The effects of temperature on embryonic and larval development. In: Wood CM, McDonald DG (eds) Global warming: implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp 177–224
- Rudneva I (2013) Stress biomarkers in fish embryos and larvae and climate changes. Biomarkers for stress in fish embryos and larvae. CRC Press, Boca Raton, pp 176–200
- Rummer JL, Couturier CS, Stecyk JAW et al (2014) Life on the edge: thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Glob Change Biol* 20:1055–1066. doi:10.1111/gcb.12455
- Sandersfeld T, Davison W, Lamare MD et al (2015) Elevated temperature causes metabolic trade-offs at the whole-organism level in the Antarctic fish *Trematomus bernacchii*. *J Exp Biol* 218:2373–2381. doi:10.1242/jeb.122804
- Sandersfeld T, Mark FC, Knust R (2016) Temperature-dependent metabolism in Antarctic fish: do habitat temperature conditions affect thermal tolerance ranges? *Polar Biol* 40:141–149. doi:10.1007/s00300-016-1934-x
- Schaefer J, Walters A (2010) Metabolic cold adaptation and developmental plasticity in metabolic rates among species in the *Fundulus notatus* species complex. *Funct Ecol* 24:1087–1094. doi:10.1111/j.1365-2435.2010.01726.x
- Schulte PM (2015) The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J Exp Biol* 218:1856–1866. doi:10.1242/jeb.118851
- Schulte PM, Healy TM, Fangue NA (2011) Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr Comp Biol* 51:691–702. doi:10.1093/icb/ucr097
- Scott GR, Johnston IA (2012) Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *Proc Natl Acad Sci USA* 109:14247–14252. doi:10.1073/pnas.1205012109/-/DCSupplemental/pnas.201205012SI.pdf
- Seebacher F, Beaman J, Little AG (2014) Regulation of thermal acclimation varies between generations of the short-lived mosquitofish

- that developed in different environmental conditions. *Funct Ecol* 28:137–148. doi:[10.1111/1365-2435.12156](https://doi.org/10.1111/1365-2435.12156)
- Sinclair BJ, Marshall KE, Sewell MA et al (2016) Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol Lett* 19:1372–1385. doi:[10.1111/ele.12686](https://doi.org/10.1111/ele.12686)
- Smith WOJ, Sedwick PN, Arrigo KR et al (2012) The Ross Sea in a sea of change. *Oceanography* 25:90–103
- Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine “winners” and “losers”. *J Exp Biol* 213:912–920. doi:[10.1242/jeb.037473](https://doi.org/10.1242/jeb.037473)
- Somero GN (2012) The physiology of global change: linking patterns to mechanisms. *Annu Rev Mar Sci* 4:39–61. doi:[10.1146/annurev-marine-120710-100935](https://doi.org/10.1146/annurev-marine-120710-100935)
- Somero GN, Devries AL (1967) Temperature tolerance of some Antarctic fishes. *Science* 156:257–258
- Stefanovic DI, Manzon LA, McDougall CS et al (2016) Thermal stress and the heat shock response in embryonic and young of the year juvenile lake whitefish. *Comp Biochem Physiol A Mol Integr Physiol* 193:1–10. doi:[10.1016/j.cbpa.2015.12.001](https://doi.org/10.1016/j.cbpa.2015.12.001)
- Strobel A, Bennecke S, Leo E et al (2012) Metabolic shifts in the Antarctic fish *Notothenia rossii* in response to rising temperature and PCO₂. *Front Zool* 9:28
- Sunday JM, Bates AE, Dulvy NK (2012) Thermal tolerance and the global redistribution of animals. *Nat Clim Change* 2:686–690. doi:[10.1038/nclimate1539](https://doi.org/10.1038/nclimate1539)
- Terblanche JS, Hoffmann AA, Mitchell KA et al (2011) Ecologically relevant measures of tolerance to potentially lethal temperatures. *J Exp Biol* 214:3713–3725. doi:[10.1242/jeb.061283](https://doi.org/10.1242/jeb.061283)
- Verberk WCEP, Bartolini F, Marshall DJ et al (2015) Can respiratory physiology predict thermal niches? *Ann NY Acad Sci* 1365:73–88. doi:[10.1111/nyas.12876](https://doi.org/10.1111/nyas.12876)
- Whitney CK, Hinch SG, Patterson DA (2013) Provenance matters: thermal reaction norms for embryo survival among sockeye salmon *Oncorhynchus nerka* populations. *J Fish Biol* 82:1159–1176. doi:[10.1111/jfb.12055](https://doi.org/10.1111/jfb.12055)
- Windisch HS, Frickenhaus S, John U et al (2014) Stress response or beneficial temperature acclimation: transcriptomic signatures in Antarctic fish (*Pachycara brachycephalum*). *Mol Ecol* 23:3469–3482. doi:[10.1111/mec.12822](https://doi.org/10.1111/mec.12822)
- Wood SN (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J R Stat Soc B* 73:3–36
- Zuur A, Ieno EN, Walker N et al (2009) Mixed effects models and extensions in ecology with R. Springer Science & Business Media, New York