COMMENTARY

The importance of incorporating natural thermal variation when evaluating physiological performance in wild species

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ABSTRACT

Environmental variability in aquatic ecosystems makes the study of ectotherms complex and challenging. Physiologists have historically overcome this hurdle in the laboratory by using 'average' conditions, representative of the natural environment for any given animal. Temperature, in particular, has widespread impact on the physiology of animals, and it is becoming increasingly important to understand these effects as we face future climate challenges. The majority of research to date has focused on the expected global average increase in temperature; however, increases in climate variability are predicted to affect animals as much or more than climate warming. Physiological responses associated with the acclimation to a new stable temperature are distinct from those in thermally variable environments. Our goal is to highlight these physiological differences as they relate to both thermal acclimation and the 'fallacy of the average' or Jensen's inequality using theoretical models and novel empirical data. We encourage the use of more realistic thermal environments in experimental design to advance our understanding of these physiological responses such that we can better predict how aquatic animals will respond to future changes in our climate.

KEY WORDS: Climate change, Environment, Growth, Metabolism, Aquatic, Ectotherm

Introduction

Aquatic environments are highly variable. For example, temperature, dissolved oxygen and salinity vary daily, and often cycle based on external forces such as tides/water flow, air temperature or sunlight. These fluctuations in physical habitat have pervasive effects on the biology of ectotherms (see Glossary) living within these environments. Temperature, in particular, has widespread effects on biological function and drives ecological patterns of ectothermic species distributions (Seebacher and Franklin, 2012). Often, physiologists study these animals under 'average' conditions (i.e. average summer temperature versus average winter temperature) to understand the effects of temperature on their physiology for practical reasons and to simplify experimental designs. However, the response to a stable average condition may be quite different from the average response to variable conditions. For example, growth rate of an animal from a stable, average environment may be different from the average growth rate of an animal from a thermally fluctuating environment. There are two related but distinct concepts potentially driving these

differences: thermal acclimation (see Glossary) to a new thermally variable environment and Jensen's inequality (see Glossary) – a mathematical property of non-linear averaging named after Johan Jensen, a Danish mathematician (Jensen, 1906). Denny (2017) recently provided an interesting Commentary on the mathematical derivation of Jensen's inequality, illustrating that in physiology, strict linear relationships between performance (e.g. growth, reproduction, metabolism) and the environment are uncommon and this will have consequences for biological systems. Thus, laboratory estimates of performance variables conducted on animals acclimated to a thermally stable environment may not accurately reflect their performance in the wild, where temperature varies. Our goal is to highlight the roles of Jensen's inequality and thermal acclimation in the physiological response of animals to thermally variable environments, particularly as it relates to aerobic metabolism as a performance indicator in fishes. We also provide empirical evidence in a wild fish to support the incorporation of thermal variability when investigating the relationship between performance and the environment.

Surprisingly, we still understand relatively little regarding the effects of thermal variation on physiological performance despite recognition of its potential importance decades ago. In 1979, Cynthia Carey wrote, 'So few studies have compared metabolic responses of ectotherms acclimated to constant and cyclic temperatures that no general patterns are apparent' (Carey, 1979). Our understanding remains incomplete almost 40 years later. That said, there has been a recent resurgence in interest to characterize these differences, particularly in terms of global climate change and conservation efforts. New theoretical models incorporating the effects of thermal variability on several performance indicators have provided insight into the effects of this common environmental condition (e.g. Denny, 2017; Dowd et al., 2015; Martin and Huey, 2008; Ruel and Ayres, 1999; Vasseur et al., 2014), but empirical tests of these models are lacking. We highlight some of these models as well as examples of physiological research incorporating natural thermal variation that support the theoretical predictions of the models. We also emphasize the importance of understanding natural temperature diel cycles in wild aquatic species.

Metabolism and temperature

Metabolism is arguably one of the most important variables in animal ecophysiology as it sets constraints on the rate of biological functions such as growth, reproduction and locomotion (Brown et al., 2004; Hochachka and Somero, 2002). Thus, measurements of metabolism – specifically, aerobic metabolic rate – are commonly used to determine species-specific optimal temperatures and to predict whole-animal performance and fitness (see Glossary). The oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis (see Glossary), proposed by H.-O. Pörtner in 2010 (Pörtner, 2010), suggests that aerobic scope, the difference between minimum and maximum aerobic metabolic rate, is maximized

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Glossary

Ectotherm
An animal whose body temperature fluctuates with its environment.

Eurythermal
Able to tolerate a wide range of temperatures.

Feed conversion
Measure of food consumed that is converted into body mass versus waste.

Fitness
Ability of an animal to reproduce within its lifetime.

Jensen's inequality
Also known as the 'fallacy of the average', this is a mathematical property of non-linear functions derived by Johan Jensen in 1906. In biology, this is used to illustrate that the response of a system to average conditions is different from the system's average response to variable conditions.

OCLTT theory
Oxygen- and capacity-limited thermal tolerance. Delivery of oxygen is the limiting factor in thermal tolerance. Proposed by H.-O. Portner, 2010 (Pörtner, 2010).

Optimum temperature
Temperature at which performance is maximal.

Parr
Juvenile freshwater salmon with distinctive 'parr' marks.

Stenothermal
Able to tolerate a narrow range of temperatures.

Thermal acclimation
A non-hereditary, usually reversible phenotypic change of an individual in response to a change in temperature.

within a defined thermal range (T_{optAS}) to optimize fitness-related performance (Pörtner, 2010; Pörtner and Farrell, 2008; Pörtner and Gutt, 2016; Pörtner et al., 2017). Outside this thermal range, aerobic scope declines and presumably decreases performance. Therefore, low aerobic scope may limit fitness-related performance such as growth, reproduction and activity (Farrell, 2009; Fry, 1947; Holt and Jørgensen, 2014; Pörtner, 2010; Wang and Overgaard, 2007). The OCLTT hypothesis is not without its limitations (Clark et al., 2013; Jutfelt et al., 2018) but has provided a link between environmental conditions, physiological performance and ecology, such that understanding an animal's physiology can allow us to predict its response to future changes in its environment (Cooke et al., 2012). Most of what we know regarding the effects of temperature on metabolism, aerobic scope and fitness in fishes comes from laboratory-based studies conducted on animals acclimated to stable thermal profiles (e.g. Brett, 1971; Claireaux et al., 2000; Clark et al., 2011; Crespel et al., 2017; Healy and Schulte, 2012; Jain and Farrell, 2003; Mazloumi et al., 2017; Norin et al., 2014; Poletto et al., 2017; Reidy et al., 2000). These studies provide important mechanistic insight – however, they do not readily allow extrapolation to natural conditions. Climate models predict that not only average temperature but also temperature variability and extreme weather events will increase (IPCC, 2013), and these are potentially as important in defining performance limits as average environmental temperature (Denny, 2017; Harley and Paine, 2009; Helmuth et al., 2005; Vasseur et al., 2014). Thus, to predict the effects of future climate change, we need to appreciate the effects of thermal variation on metabolism, thermal performance curves (TPCs) and TPC plasticity. By incorporating Jensen's inequality, we can begin to understand the effects of thermal variation on mean trait values (i.e. growth rate) and overall fitness.

Jensen's inequality and physiological performance

TPCs are often generated to evaluate and estimate the ecological consequences of temperature. These curves measure instantaneous performance traits across fixed temperatures (Huey and Slatkin, 1976) and do not consider natural field conditions that are often heterogeneous in space and time (Sinclair et al., 2016). One way we can use TPCs and partially skirt around this temporal and spatial dynamism that is implicit in these curves is to incorporate Jensen's inequality. Here, using a TPC for a eurythermal animal (see Glossary; Fig. 1), generated with the assumption that animals are acclimated to constant temperature for each performance measurement, we can calculate the performance (y) at any given temperature (x) using $y=f(x)$. As Denny (2017) describes, if the relationship between x and y is a non-linear function, as it often is in biology, the average of y at x is not equal to the y of average x. In this example, the optimum temperature (x; see Glossary) that maximizes performance (T_{opt}) is 18°C (Fig. 1). If temperature fluctuates about this average, such that equal time is spent at two different temperatures on the curve, we can calculate the performance at the average temperature (average x) using the slope of the linear line connecting the two points (Denny, 2017; Fig. 1). For example, using a range of ±2°C (16–20°C, average 18°C; line a), according to Jensen's inequality, the performance at average 18°C is roughly 8% lower than the average performance at a stable 18°C. While this difference is relatively minor, it becomes magnified as the thermal range increases (±3°C, line b, 15% lower; ±4°C, line c, 30% lower). This difference is further exacerbated if examined in a stenothermal (see Glossary) animal whose temperature range is comparatively narrow (i.e. a steeper TPC). In this instance, small temperature variations dramatically increase the effect of Jensen's inequality, resulting in larger decreases in physiological performance. Furthermore, given that most TPCs are asymmetric and skewed towards the critical minimum temperature (CT_{min}), changes in temperature beyond the optimum and approaching the critical maximum temperature (CT_{max}) will have comparatively greater performance effects than the same change in temperature below the optimum. Thus, thermal variability is crucial in predicting how an ectotherm will respond to changes in temperature.

Given the shape of the TPC (Fig. 1), changes in temperature can have negligible, small or large effects on performance (Sinclair et al., 2016). Indeed, the effect of Jensen's inequality on animal performance is dependent on several factors: (1) the range of temperatures at which the species can survive (i.e. thermal breadth), (2) the nature of the relationship (e.g. exponential, logarithmic, linear), (3) the skewness of the relationship and (4) the range of the thermal variation with respect to the shape of the curve (Fig. 1). For example, in cases of thermal variation near the T_{opt}, performance is predicted to be lower under fluctuating conditions (Fig. 1, lines a, b and c), with a greater inequality for stenothermal than eurythermal animals. By contrast, if, in this example, the thermal range being tested is at the colder end of the TPC (e.g. 6–12°C), performance is predicted to be higher under fluctuating compared with stable conditions (Fig. 1, line d). When thermal variation spans linear sections of the curve, there is no effect of Jensen's inequality.

This predicted difference in performance with TPCs does not necessarily imply that animals from stable or variable environments have different trait or performance values when both are measured at T_{opt}. Rather, the predicted decrease in average performance of animals in thermally variable environments results from the acute effects of exposure to temperatures above and below T_{opt} that would alter performance over time. However, many animal species also
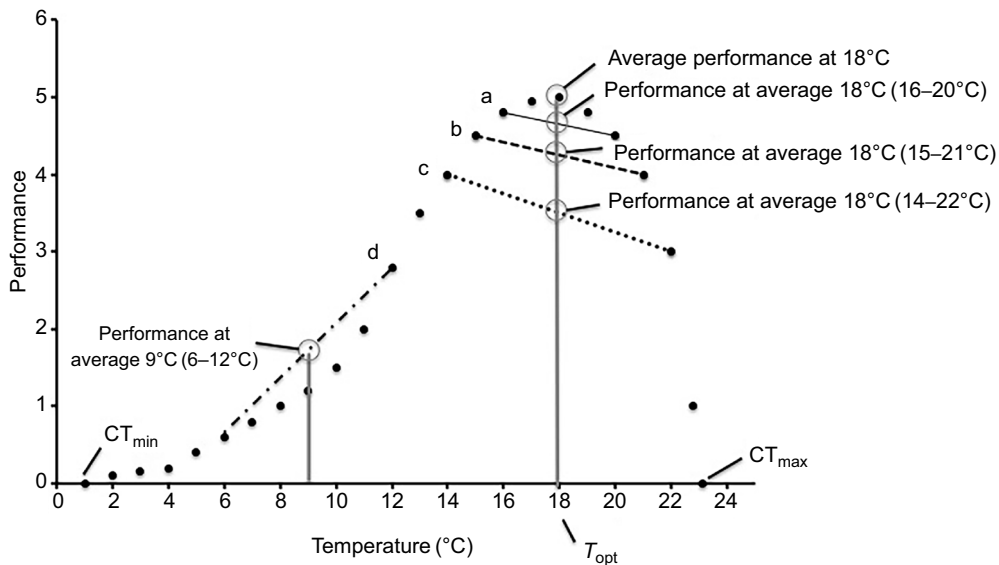
Fig. 1. The effects of Jensen's inequality on a theoretical thermal performance curve. The theoretical thermal performance curve (TPC; dots) was modified from Denny (2017). The optimum temperature (T_{opt}), where performance is maximized, is 18°C (vertical grey line), and the average performance is indicated. When the temperature varies near T_{opt} from 16 to 20°C, the performance at the average temperature (18°C) is predicted to be lower (line a) than the average performance at the stable temperature of 18°C. As variability increases (15–21°C, 14–22°C), performance continues to decline (lines b and c). In contrast, when temperature varies at the colder end of the curve (6–12°C), the performance at the average temperature (9°C) is predicted to increase (line d). CT, critical thermal minimum (min) or maximum (max).

exhibit varying degrees of plasticity in their TPCs when exposed to chronic thermal change in order to maximize or maintain performance under new variable environmental conditions (Angilletta, 2009; DeWitt and Scheiner, 2004; Guderley and St-Pierre, 2002; Hochachka and Somero, 2002; Wilson and Franklin, 1999). In such cases, ectothermic animals can reduce the thermal sensitivity of important physiological performance traits such as metabolic rate, enzyme activity, heart rate, etc. In turn, this decreased sensitivity should widen thermal breadth and buffer against temperature variability such that performance is maintained.

Recent theoretical performance models incorporating thermal variation have shown that variation is as strong, or stronger, than average temperature alone at predicting future fish performance in forecasted climate scenarios (Vasseur et al., 2014). Vasseur et al. (2014) developed a theoretical model to account for both average temperature and temperature variability and tested it against 38 previously developed species-specific TPCs for globally distributed ectothermic invertebrates under projected climate model temperature extremes and variation. Using the average temperature alone, only 32% of the variation in thermal performance could be explained; however, when temperature variability was used, it explained 54% of the variation. Remarkably, 93% of the variation in performance can be explained if both average and variance are included in the model (Vasseur et al., 2014). The inequality between the performance at an average temperature and the average performance across a range of temperatures is not necessarily a biological manifestation; rather, it is a mathematical consequence of the non-linearity of TPCs and the natural variation of temperature in the wild. Thus, any predictive understanding of ectotherm physiological performance in future climate scenarios must account for thermal variability.

Empirical data
Empirical evidence is now emerging that supports these predicted differences in performance between animals in stable and thermally variable environments. Recent studies in fishes, reptiles and insects have shown that physiological responses of thermally cycled animals are distinct from those of animals acclimated to stable temperatures. We present data highlighting the differences between the effects of Jensen's inequality on mean trait values (e.g. growth rate) and TPC plasticity on instantaneous measures of physiological performance (e.g. metabolic rate).

Growth rate
To date, the majority of data suggest that thermal variability will decrease growth rate, as predicted by the theoretical models discussed above. In salmonid fishes, growth rate is significantly lower when fish are exposed to thermal fluctuations compared with stable temperatures (Flodmark et al., 2004; Imholt et al., 2011; Meeuwig et al., 2004). Furthermore, both Imholt et al. (2011) and Meeuwig et al. (2004) observed that growth rate in Atlantic salmon (*Salmo salar*) and cutthroat trout (*Oncorhynchus clarki henshawi*) decreased further as the magnitude of temperature fluctuations increased, even if the daily average temperature remained the same. A similar pattern was observed for a variety of North American temperate fish species, although not all responded in the same way (Eldridge et al., 2015). Growth rate also decreased in spike dace (*Meda fulgida*) exposed to thermal variations of 24–34, 28–34 and 30–34°C (Carveth et al., 2007). Similarly, the growth of juvenile walleye (*Sander vitreus*) decreased, whereas the growth of adult perch (*Perca flavescens*) increased when exposed to thermal variation (23 versus 23±4°C) (Coulter et al., 2016), highlighting the species-specific effects of thermal variation on growth. It is possible that life stage also played a role in these differences, as well as their individual thermal optima (22°C for walleye, 25°C for perch; Hokanson, 1977). Comparing these data with species-specific TPCs may help to reconcile these differences, bearing in mind the often static, instantaneous nature of the TPC. These changes in growth rate with thermal variation highlight the effects of Jensen's inequality such that the change in average growth rate is the result of changes in instantaneous growth across the range of temperatures experienced by the fish. The magnitude of these changes is dependent on the overall shape of the TPC and the range of temperatures being tested.

Niehaus et al. (2012) attempted to validate the framework for predicting performance during thermal variation with empirical data on larval striped marsh frogs (*Limnodynastes peronii*). Developmental and growth rates were estimated from a model of thermal reaction norms based on stable acclimation temperatures, and then compared with empirical measurements under natural fluctuating temperature conditions. In the majority of cases, the observed and predicted rates were significantly different, and this difference increased with increasing thermal variation (18–28 versus 18–34°C; Niehaus et al., 2012). TPC plasticity may

account for some of these differences as the animals acclimate to their new thermal cycle. These findings further underscore the inability to predict the effects of thermal variability on growth performance using stable temperature models alone.

Metabolism

Changes in growth rate are probably a result of changes in metabolic rate, food intake or feed conversion/growth efficiency (see Glossary). For example, food intake decreased in brown trout (*Salmo trutta*) held in a diel thermal cycle (Flodmark et al., 2004). However, there was no difference in food intake in Atlantic salmon (Imholt et al., 2011) or cutthroat trout (Meeuwig et al., 2004) in variable thermal environments, despite a decrease in growth. Similarly, no differences in food intake or gross feed conversion were observed in brown trout grown under varying thermal regimes compared with a stable temperature (Spigarelli et al., 1982). The effects of thermal variability on food intake and conversion are relatively understudied, but these few examples suggest that they may not play a significant role in the observed changes in growth rate. However, this is a complex area of research given that feed composition, digestive physiology, temperature and time of day all play a role in feed conversion.

It is possible that decreases in growth rate in thermally variable environments may be due to changes in routine metabolic rate (RMR) as a result of acclimation (Beauregard et al., 2013). RMRs (fasted, unrestrained, but minimal activity) of Atlantic salmon parr (see Glossary) exposed to 20±2 or ±3°C were 25% and 32% higher, respectively, than those of parr maintained at a constant 20°C, when measured at 20°C (Beauregard et al., 2013). Interestingly, there were no changes in standard metabolic rate (SMR; RMR in the absence of any spontaneous activity) for Atlantic salmon at 15±2.5°C when compared with that at a stable 15°C. However, at 20±2.5°C there was a 33% increase in SMR compared with that at a stable 20°C, when measured at 20°C (Oligny-Hébert et al., 2015). As these are measures of instantaneous $\dot{M}_{O_2}$ at a single defined temperature, the effects of thermal variability will be dependent on the capacity for acclimation and probably the result of changes in the shape of individual TPCs after exposure to fluctuating temperatures. A similar pattern has also been observed in toads, *Bufo boreas* sp. (Carey, 1979). An increase in RMR presumably would reduce energy for growth and help explain the decrease in growth rate with thermal variability. However, the growth rate of tadpoles of the striped marsh frog *Limnodynastes peronii* was decreased under

thermally variable conditions, but there were no corresponding changes in RMR (Kern et al., 2015). Similarly, there was no change in RMR of this species with increased thermal variability; however, growth rate was decreased (Niehaus et al., 2011), suggesting that there may not have been any changes in the TPC, but the effects of Jensen's inequality remain apparent in the growth rate. The effects of thermal variability on the shape of individual species' TPCs probably alters instantaneous measures of RMR and other rate functions (i.e. heart rate, enzymatic rates, etc.) as they are variable across a variety of species: *Panopeus herbstii* and *Uca pugilator* (Dame and Vernberg, 1978), spiders (*Geolycosa godeffroyi*; Humphreys, 1975), mussels (*Mytilus edulis*; Widdows, 1976). Such variation further supports the need for more research on the metabolic effects of thermal variability. It will be important to understand these differences in terms of both Jensen's inequality (mean trait value over time, e.g. growth rate) and acclimation (e.g. TPC plasticity), where the latter may modify instantaneous measurements of performance such as metabolic rate.

Atlantic salmon: a case study

Given the effects of thermal variability on RMR in the above examples, we sought to understand how thermal variation would affect both RMR and maximal metabolic rate (MMR) and aerobic scope of wild juvenile Atlantic salmon parr from the Miramichi River in NB, Canada (see Box 1 for methods). We predicted that RMR would increase, thereby decreasing aerobic scope. We did observe a decrease in aerobic scope (Fig. 2C) in salmon acclimated to 16–21°C compared with stable 18.5°C; however, this was not due to an increase in RMR. Rather, both RMR and MMR decreased significantly (Fig. 2A,B). These metabolic changes are most likely due to alterations of the shape of the TPC (i.e. acclimation) in those fish acclimated to a thermally variable environment. As the shape of the TPC changes, instantaneous measures of $\dot{M}_{O_2}$ at a single temperature would also change. To determine whether there was any effect of Jensen's inequality, one would have to measure $\dot{M}_{O_2}$ at each temperature within the thermal cycle and calculate the average $\dot{M}_{O_2}$ from those individual values.

The 'plastic floor and concrete ceilings' concept suggests that basal (floor) performance measurements have considerably more plasticity for thermal acclimation than do maximal (ceiling) performance measurements (Sandblom et al., 2016), and this may partially account for the variability in basal responses within or between species exposed to thermal variation that we have highlighted. TPCs for basal performance may shift more readily
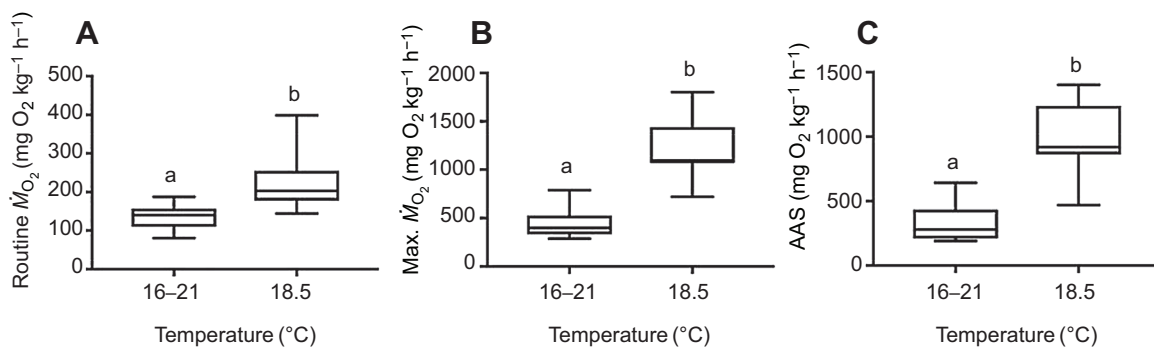
Fig. 2. Routine and maximum metabolic rate ($\dot{M}_{O_2}$), and aerobic scope of Atlantic salmon acclimated to a diel thermal cycle of 16–21°C (*n*=14) or stable 18.5°C (average of the cycle; *n*=13) for 3 weeks. (A) Routine $\dot{M}_{O_2}$ (RMR); (B) maximum $\dot{M}_{O_2}$ (MMR); and (C) absolute aerobic scope (AAS). Boxes represent the 25th and 75th percentile and the horizontal line within the box is the median. Whiskers are the minimum and maximum values. Different letters denote significant differences (*P*<0.001) between temperature groups.

Box 1. Methods

Experimental animals

Juvenile wild Atlantic salmon (*Salmo salar* Linnaeus 1758) parr (26.2± 1.2 g) were electrofished from the Rocky Brook tributary, which is part of the South West Miramichi river system, in New Brunswick, Canada (Fig. 1). Fish were held in 300 l tanks in a 1869 l recirculating freshwater system at 16°C with a natural photoperiod and were fed once daily *ad libitum* with dry pellets (Corey Nutrition Company, Fredericton, NB, Canada; 1.0 mm) in the Crabtree Aqualab at Mount Allison University for 4 weeks prior to experimentation. The Mount Allison Animal Care Committee approved all procedures following the Canadian Council on Animal Care guidelines.

Experimental setup

Fish were acclimated to two different temperature protocols throughout the experiment: 16–21°C (*n*=14) and 18.5°C (*n*=13). These temperatures were chosen to reflect a natural diel temperature cycle in the Miramichi River (16–21°C) (Caissie et al., 2012) or the average temperature of the diel cycle (18.5°C). The 16–21°C acclimation was set on a 12 h cycle: 16°C at 07:00 h and 21°C at 19:00 h and a ramp rate of ~0.42°C h^{-1}. Throughout the experiment, fish continued to be fed *ad libitum* once daily, but feeding was ceased 24 h prior to experimentation. Experiments were conducted on the same group of fish after acclimation to each temperature regime for at least 3 weeks. Fish were first exposed to 16–21°C for 3 weeks, then 18.5°C for 3 weeks. It is important to note that these same fish were used in a previous, separate, experiment in which they were swum in the respirometer. Therefore, in the current experiment, all fish had had prior experience in the swim tunnel, thereby limiting any training effect throughout the various acclimations.

Experimental protocol

All metabolic rate measurements and critical swimming speed tests were conducted in a swim tunnel respirometer (Loligo Systems) consisting of a 30 l measurement chamber submerged in a 120 l aerated water bath similar to that used by Tunnah et al. (2016). Testing took place between 08:00 h and 10:00 h for all trials at a common temperature (16°C) for all groups regardless of acclimation temperature. Fish acclimated to 16–21°C or 18.5°C in their holding tanks experienced a matched temperature of the respirometer during the overnight acclimation. For the fish in the 16–21°C group, the water temperature in the respirometer was heated to match that in the holding tank (21°C at 19:00 h) and set to cool overnight from 21°C to 16°C (07:00 h) to mimic the thermal cycle of the holding tank. For the fish held at 18.5°C, the water was kept at 18.5°C overnight and, at 07:00 h, the water temperature was dropped to 16°C and held at this temperature for at least 1 h prior to experimentation.

Aerobic metabolic rate

Individual fish were removed from their holding tank and measurements were taken of mass and length before experimentation. Fish were not anaesthetized before measurements to reduce recovery time, and this procedure was completed in under 1 min. Fish were immediately placed into the respirometer with constant flow (10 cm s^{-1}) and left overnight (minimum of 8 h) in the dark to acclimate and return to a resting state. Routine mass-specific metabolic rate ($\dot{M}_{O_2}$) was measured using intermittent closed-loop respirometry. Briefly, the decline in dissolved O_2 was measured every 10 min for 300 s. Between each measurement, the internal chamber was flushed with fresh oxygenated water to maintain the dissolved O_2 above 90% air saturation throughout the experiment. Routine $\dot{M}_{O_2}$ was calculated from the average of the lowest 10 oxygen consumption rates in the morning prior to experimentation, taking into account the volume of the fish, volume of the chamber, temperature and barometric pressure. To account for any possible bacterial oxygen consumption in the respirometer, a blank trial was run with no fish for 8 h during each of the three trials. It was found that bacterial oxygen consumption was negligible and was therefore not used to correct the data. Maximum $\dot{M}_{O_2}$ was measured immediately after the critical swimming speed test (U_{crit}) (Jain and Farrell, 2003) when fish were fully exhausted. O_2 depletion was continuously monitored in 30 s increments immediately after the U_{crit} test for 5 min, or until the rate of O_2 consumption began to slow down as the fish recovered. Maximum $\dot{M}_{O_2}$ was calculated from the largest O_2 consumption rate during this period.

Statistical analysis

All statistical analyses were performed in RStudio (v 2.0.243). Because fish were repeatedly sampled at each temperature, an error of independence arose, resulting in inflated degrees of freedom. To account for this error, the α-level was set to 0.01 and a *t*-test used to assess statistical differences between different temperature acclimations.

than TPCs for maximal performance, ultimately altering the 'scope' for these activities. Furthermore, it has been suggested that the mean temperature of a fish's origin river may also play a role in determining SMR (Eliason et al., 2011; Farrell et al., 2008; Healy and Schulte, 2012). Thermal acclimation to specific environments across generations adds another layer of complexity when investigating wild populations (Sandblom et al., 2016) and will probably play a role in their response to thermal variability.

The OCLTT hypothesis predicts that a decrease in aerobic scope will decrease performance, and this is true for many, but not all, species, at stable temperatures (Donelson et al., 2014; Grans et al., 2014; Healy and Schulte, 2012; Khan et al., 2014; Norin et al., 2014; Speers-Roesch and Norin, 2016). Considering Jensen's inequality, TPC plasticity and our preliminary data on wild salmon, there is a clear need to understand the relationship between aerobic scope and other performance indicators in thermally variable environments. In particular, measuring important life history traits such as growth, locomotion, foraging ability and reproduction will help to clarify the effects of thermal variability on overall fitness. Individual changes in fitness can potentially lead to community- or population-level effects that will significantly alter species abundance and/or distribution.

Indicators of stress

There have been several studies investigating whether and how natural temperature variation affects molecular markers of thermal stress (e.g. Todgham et al., 2006; Fangue et al., 2011; Narum and Campbell, 2015). However, until recently, the underlying cellular mechanisms governing differences in whole-animal metabolic rate have been relatively understudied. Again, using wild Atlantic salmon as a model, we determined that short-term warming thermal fluctuations increase expression of heat shock protein 70 (HSP70) protein (Corey et al., 2017; Tunnah et al., 2016) – an important adaptive response to thermal stress that helps maintain the structure and function of cellular proteins. The energetically costly production and breakdown of HSPs could further impact metabolism and the effects of thermal variation (Paaijmans et al., 2013). Given the expected decrease in aerobic metabolic rate in a thermally variable environment, it would be reasonable to assume that there will be stress-related shifts in metabolic pathways, potentially towards anaerobic metabolism, at least in the short term, when the temperature varies. At present, there is limited information on metabolic pathways or their metabolites during thermal variation; however, we have shown that short-term (<5 days) exposure to diel thermal cycles results in changes in metabolites (lactate, glucose, glycogen) and regulatory pathways controlling

metabolism (AMPK, Raptor) (Callaghan et al., 2016; Corey et al., 2017; Tunnah et al., 2016). Notably, Callaghan et al. (2016) showed that short-term thermal cycling initially induced a catabolic response, as one would expect during periods of stress, but as thermal cycling continued, energetically expensive processes such as protein synthesis were reactivated and energy stores recovered. This suggests that fish may be able to remodel their metabolism in the face of thermal variability to maintain some capacity to cope with future physiological or environmental stresses.

Future directions

The effects of thermal variability on thermal preference and behaviour should also be evaluated. Most ectotherms use behavioural mechanisms (where possible) to regulate body temperature in order to maximize performance or avoid critical temperatures where performance declines (e.g. Martin and Huey, 2008, and references therein). However, because TPCs are typically asymmetric, increases in temperature above T_{opt} will decrease performance more so than a decrease in temperature. Therefore, in a thermally variable environment, where animals may be frequently exposed to temperatures well above T_{opt}, they may prefer a temperature below T_{opt} (where performance is not fully maximized) to maximize performance over the long term (Martin and Huey, 2008).

Temperature, of course, is not the only environmental factor that varies in nature. For example, oxygen, salinity and pH can change diurnally in many ecosystems (Baumann et al., 2015), yet we have very limited physiological data on the effects of diel variation of these factors (Cone, 1988; Dan et al., 2014; Yang et al., 2013). Individually, we can begin to determine how animals respond to variation of each of these factors, with the goal of developing a comprehensive framework to explain the effects of multiple covariates on animal performance. At present and to our knowledge, there is only one theoretical model that can predict the effects of simultaneous variation of multiple environmental factors on performance – however, the time scale or periodicity is not included (Koussoroplis et al., 2017). It is presently unclear how the duration of variation in environmental factors might affect biological functions, and this is clearly a rich area for future study.

The limited availability of cellular- and molecular-level data is hampering the development of a convincing mechanistic hypothesis to explain the predicted and observed whole-animal changes in growth and other performance indicators with natural thermal variation. Going forward, it will be imperative to understand the effects of thermal variation at all levels of biological organization so we can reliably predict the impact of climate change on ectotherms. Given that growth is affected by thermal variation, we can reasonably assume that other life history processes such as reproduction or locomotion will be as well, translating into potentially major population-level effects. Predicting the physiological responses of ectotherms to climate change using TPCs will provide a broad-spectrum interpretation; however, we must be aware of their assumptions and limitations (see Sinclair et al., 2016, for a review). As suggested by Sinclair et al. (2016), incorporating real-world issues and (multiple) environmental conditions when evaluating an ectotherm's fitness in a given environment will inform and advance our predictive capacity regarding climate change.

Experimental designs to understand the effects of thermal variability on aspects of performance will vary depending on the experimental question and species investigated. Studies to evaluate the effects of both TPC plasticity and Jensen's inequality, individually and in combination, will be required to better appreciate the effects of

thermal variation on animal performance. One may predict that species that are less thermally plastic may be more susceptible to the effects of Jensen's inequality resulting from thermal variation and, thus, more susceptible to the effects of climate change.

Concluding remarks

Historically and understandably, physiologists often remove natural variability in lab studies, relying on 'average' conditions in order to more clearly understand biological processes. Using this paradigm, we have made great strides in our knowledge of the effects of temperature on physiological processes and have developed mechanistic and predictive models in an attempt to understand how animals will respond to a changing climate. We are now in a position where we can no longer ignore the natural variation in temperature (and other variables) and must seek to understand its effects on animals. We have provided examples here where growth and metabolism are different in animals exposed to thermal variation, and provide predictions based on Jensen's inequality as to the extent and directionality of these differences. We encourage the use of realistic environmental temperature profiles in experimental biology to increase our knowledge and understanding of these differences so we can create more realistic predictions about the effects of climate change on animals.

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