


# The ecological relevance of critical thermal maxima methodology for fishes

Jessica E. Desforges<sup>1</sup>  | Kim Birnie-Gauvin<sup>2,3</sup> | Fredrik Jutfelt<sup>4</sup> |  
 Kathleen M. Gilmour<sup>5</sup> | Erika J. Eliason<sup>3</sup> | Terra L. Dressler<sup>3</sup> |  
 David J. McKenzie<sup>6</sup> | Amanda E. Bates<sup>7</sup> | Michael J. Lawrence<sup>8</sup> | Nann Fangue<sup>9</sup> |  
 Steven J. Cooke<sup>1</sup>

<sup>1</sup>Department of Biology and Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, Ontario, Canada

<sup>2</sup>Department of Ecology, Evolution & Marine Biology, University of California Santa Barbara, Santa Barbara, California, USA

<sup>3</sup>Section for Freshwater Fisheries and Ecology, National Institute of Aquatic Resources, Technical University of Denmark, Silkeborg, Denmark

<sup>4</sup>Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

<sup>5</sup>Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

<sup>6</sup>MARBECC, Université Montpellier, Montpellier, France

<sup>7</sup>Department of Biology, University of Victoria, Victoria, British Columbia, Canada

<sup>8</sup>Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

<sup>9</sup>Department of Wildlife, Fish, and Conservation Biology, University of California Davis, Davis, California, USA

## Correspondence

Jessica E. Desforges, Department of Biology and Institute of Environmental and Interdisciplinary Science, Carleton University, 1125 Colonel By Dr., Ottawa, ON K1S 5B6, Canada.

Email: [jdesforg@gmail.com](mailto:jdesforg@gmail.com)

## Funding information

NSERC Discovery grant

## Abstract

Critical thermal maxima methodology (CTM) has been used to infer acute upper thermal tolerance in fishes since the 1950s, yet its ecological relevance remains debated. In this study, the authors synthesize evidence to identify methodological concerns and common misconceptions that have limited the interpretation of critical thermal maximum ( $CT_{max}$ ; value for an individual fish during one trial) in ecological and evolutionary studies of fishes. They identified limitations of, and opportunities for, using  $CT_{max}$  as a metric in experiments, focusing on rates of thermal ramping, acclimation regimes, thermal safety margins, methodological endpoints, links to performance traits and repeatability. Care must be taken when interpreting CTM in ecological contexts, because the protocol was originally designed for ecotoxicological research with standardized methods to facilitate comparisons within study individuals, across species and contexts. CTM can, however, be used in ecological contexts to predict impacts of environmental warming, but only if parameters influencing thermal limits, such as acclimation temperature or rate of thermal ramping, are taken into account. Applications can include mitigating the effects of climate change, informing infrastructure planning or modelling species distribution, adaptation and/or performance in response to climate-related temperature change. The authors' synthesis points to several key directions for future research that will further aid the application and interpretation of CTM data in ecological contexts.

## KEYWORDS

climate change, ectotherms, temperature, thermal ecology, thermal stress, upper thermal tolerance

# 1 | INTRODUCTION: THE HISTORY OF CTM IN FISHES, PAST APPLICATIONS AND LIMITATIONS

Since its development in 1944 (Cowles & Bogert, 1944), scientists have used critical thermal maxima methodology (CTM; see Box 1 for glossary) as a way to obtain a proxy for upper thermal tolerance in organisms. The temperature at which an organism reaches a critical endpoint ( $CT_{max}$ ) has become a fundamental metric in fish ecology used to understand the impacts of thermal stress on performance, physiology and behaviour and to forecast potential impacts of climate warming on distribution, acclimation capacity and life-history strategies of fishes. Historically,  $CT_{max}$  (critical thermal maximum) was defined as “the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death” (Cowles & Bogert, 1944). The simplicity of measuring  $CT_{maxima}$ , along with consistent behavioural responses at upper thermal limits across diverse taxa, has made CTM a popular choice in fisheries science since its inception (reviewed in Lutterschmidt & Hutchison, 1997). Indeed, CTM assisted in the establishment of regulatory guidelines to manage thermal pollution from anthropogenic sources (e.g., United States Environmental Protection

Agency, 2022; Holland *et al.*, 1974). As novel applications emerged, the original definition of  $CT_{max}$  evolved to include specifications regarding the importance of using consistent and acute heating rates, as well as uniform fish size, duration and temperature of the acclimation period and consideration for the significance of thermal history (Lutterschmidt & Hutchison, 1997). Unfortunately, the attempted refinement of CTM over time gave rise to a wide range of methods used to derive empirical estimates of  $CT_{max}$ , which have led to inconsistencies across studies that hinder the applications of CTM in certain contexts (Becker & Genoway, 1979; Lutterschmidt & Hutchison, 1997; Pottier *et al.*, 2022).

The most widely accepted definition of  $CT_{max}$  includes guidelines to achieve an acute rate of thermal ramping (typically  $0.3^{\circ}\text{C min}^{-1}$  or  $18^{\circ}\text{C h}^{-1}$ ) and a standardized endpoint marked by loss of equilibrium (LOE; Becker & Genoway, 1979). LOE is one of the most prevalent responses to thermal stress (Lutterschmidt & Hutchison, 1997) and is used as a common (and non-lethal) endpoint for CTM testing. Following a recommended acute rate of thermal ramping to evaluate  $CT_{max}$  is critical for two main reasons: (a) it controls for discrepancies in temperatures between the water and the internal body of the fish and (b) it prevents the modulation of physiological or biochemical pathways involved in inducing acclimation responses (Becker & Genoway, 1979; Beiting

## BOX 1 Glossary

Term	Definition
$CT_{max}$	Critical thermal maximum refers to a value for an individual fish during one trial.
$CT_{maxima}$	Critical thermal maxima (plural) is the “arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized, and the animal loses its ability to escape from conditions that will promptly lead to its death when heated from a previous acclimation temperature at a constant rate just fast enough to allow deep body temperatures to follow environmental temperature without a significant time lag” (Cox, 1974) or simply the mean of $CT_{max}$ values obtained from a group of fish.
CTM	Critical thermal maxima methodology.
Thermal performance	Individual response to changes in temperature, measured with physiological or behavioural indices.*
Thermal tolerance	The thermal threshold that an individual can sustain. This can be measured using a variety of physiological or behavioural indices.*
Acute	Characterizes short-term responses, from seconds to hours.*
Chronic	Characterizes long-term responses, from days to years.*
Ecological relevance	The degree to which a concept or method can be applied to ecological contexts while deriving impactful insights.*
Thermal safety margins	Either defined as the difference between acclimation temperature and $CT_{max}$ or the difference between the environmental temperature and $CT_{max}$ .
Acclimation	Changes in biochemical pathways and molecules that allow for a new stable physiological state (typically days to weeks).
Resistance	Short-term responses to environmental changes such as altering the production of heat-shock proteins, switching to anaerobic metabolism or seeking cooler refuges (Bates & Morley, 2020).*
Repeatability	Consistency of an individual's performance over longer time scales, measured by quantifying the proportion of total variation of a trait that is due to differences between individuals (Dohm, 2002).
Phenotypic Plasticity	The potential for an organism to produce a range of different, relatively fit phenotypes in multiple environments (DeWitt <i>et al.</i> 1998).

Note. \*indicates the operational definition used for the purposes of this paper.

*et al.*, 2000; Lutterschmidt & Hutchison, 1997; Mora & Maya 2006). Nonetheless, many studies used different CTMs despite previous efforts to standardize methods; across studies, heating rates were found to vary from  $0.041^{\circ}\text{C h}^{-1}$  to  $3.8^{\circ}\text{C min}^{-1}$  (equivalent to  $1.0^{\circ}\text{C day}^{-1}$  to  $5472.0^{\circ}\text{C day}^{-1}$ ), whereas in some studies, the heating rates were not reported at all (Lutterschmidt & Hutchison, 1997). These methodological differences limit the ability to interpret and generalize results of  $\text{CT}_{\text{max}}$  in broader contexts.

Although a constant rate of temperature increases controls for some variation across CTM, it does not account for morphological and physiological differences among fishes. Consequently, research has begun to question the validity of using a standardized ramping rate ( $0.3^{\circ}\text{C min}^{-1}$ ) across all fish species (Jutfelt *et al.*, 2019). Significant temperature differentials have been measured between water temperature and core tissue temperatures in numerous species, including zebrafish (Morgan *et al.*, 2018), perch (Sandblom *et al.*, 2016) and cod (Jutfelt *et al.*, 2019). Universally using a uniform rate of  $0.3^{\circ}\text{C min}^{-1}$  can lead to unrealistically high estimation of thermal limits for larger fishes due to large thermal inertia in relation to body surface area (Fangue *et al.*, 2011; Jutfelt *et al.*, 2019). Correcting the rate of thermal ramping to account for fish size or morphological differences could, therefore, provide a better representation of thermal limits in fish. Methodological inconsistencies in measuring upper thermal tolerance across life stages have also led to much debate on the relevance of CTM (Dahlke *et al.*, 2020, 2022; Pottier *et al.*, 2022). Dahlke *et al.* (2020) found that embryos and breeding adult fishes are much more susceptible to temperature change than conspecifics in other life stages. Nonetheless, a response by Pottier *et al.* (2022) recently suggested that the analyses performed by Dahlke failed to account for methodological variations, further exemplifying the importance of deriving comparable estimates in generating reliable conclusions from multiple studies.

Standardizing a physiological endpoint (*i.e.*, LOE) has similar limitations to a constant rate of ramping. We know little about the underlying physiological mechanism (or combination of mechanisms) that results in loss of function at high temperatures (*e.g.*, Ern *et al.*, 2016, 2017; Jutfelt *et al.*, 2019; Lefevre *et al.*, 2021; Wang *et al.*, 2014). For instance, morphological or physiological differences in study organisms could alter the LOE response and lead to over- or underestimated  $\text{CT}_{\text{max}}$  values. Fish of different age classes can respond differently to thermal ramping due to past thermal exposure (*e.g.*, previous exposure to thermal extremes or lack of extremes; Morgan *et al.*, 2018).  $\text{CT}_{\text{max}}$  can differ between sexes, across populations, with diet and size (Isaza *et al.*, 2019; Kumar *et al.*, 2016; McKenzie *et al.*, 2020; O'Donnell *et al.*, 2020; Zhang & Kieffer, 2014).  $\text{CT}_{\text{max}}$  may also vary under different pH, salinity and dissolved oxygen concentration regimes (*e.g.*, Ern *et al.*, 2016; Madeira *et al.*, 2014; Potts, 2020).

In summary, although CTM is often perceived as a straightforward method to infer thermal tolerance, complex interactions exist when the experimental design deviates from the fundamental concepts of acclimation, rate of thermal ramping and a repeatable, non-lethal endpoint. In addition, thermal limits are inherently linked to the environment, morphology, genetics and physiology, presenting

confounding effects that have yet to be fully elucidated. In this study, the authors focus specifically on assessing the ecological relevance of using  $\text{CT}_{\text{max}}$  as a metric of thermal tolerance in fishes. In the following section, they present a series of questions regarding CTM and its ecological relevance. They also review important considerations for measuring and using  $\text{CT}_{\text{max}}$  in ecologically relevant ways, as presented in Table 1, and address how issues that arise during CTM can be avoided. They then highlight how CTM research can be integrated as a tool to describe individual, population, community and ecosystem-level responses to progressive warming and increasingly variable environments. They conclude by providing considerations that should be incorporated into future studies in an effort to increase the applicability of  $\text{CT}_{\text{max}}$  in fish ecology and by providing key directions for future research.

## 2 | EVALUATING THE ECOLOGICAL RELEVANCE OF $\text{CT}_{\text{MAX}}$ AS A MEASURE OF UPPER THERMAL TOLERANCE

### 2.1 | Is the rate of thermal ramping important when designing experiments?

One of the most common criticisms of CTM is directed towards the use of rapid rates of thermal ramping that are rarely observed in the wild (*e.g.*, Chown *et al.*, 2009; Terblanche *et al.*, 2007). Time is an important factor mediating responses to thermal challenges (*i.e.*, for how long and how fast organisms are exposed to thermal challenges), yet this aspect of thermal tolerance is often ignored when explaining physiological and biological limits (see Bates & Morley, 2020; Lefevre *et al.*, 2021). During fast rates of warming (seconds or minutes), organisms respond to thermal stress by modulating neural and endocrine mechanisms, such as increased adrenergic stimulation and corticosteroid secretion, increased ventilation, heart rate and cardiac output (Ekström *et al.*, 2014, 2019; Saravia *et al.*, 2021).  $\text{CT}_{\text{max}}$  may thus reflect the thermal tolerance of immediately critical organs, such as the brain and heart (Ekström *et al.*, 2018; Jutfelt *et al.*, 2019). Physiological mechanisms underlying LOE in fishes are not well understood (*e.g.*, Ern *et al.*, 2016, 2017; Jutfelt *et al.*, 2019; Lefevre *et al.*, 2021; Wang *et al.*, 2014), and different biological pathways may be involved in coping with acute vs. chronic thermal stress (Bates & Morley, 2020; Lefevre *et al.*, 2021; Peck, 2011). Therefore, it is important not to over-interpret  $\text{CT}_{\text{max}}$  as the only indicator of thermal tolerance, thermal performance or thermal acclimation potential.

When thermal ramping occurs at relatively slow rates (over several days to months), organisms can undergo acclimation, which refers to changes in biochemical pathways and molecules that allow for a new stable physiological state (Bates & Morley, 2020). Chronic thermal stress (days, weeks and months) can be described by responses such as cessation of feeding, decreased growth rates or increased vulnerability to predation (Jutfelt *et al.*, 2021), none of which are typically considered in CTM. Indeed, some researchers argue that  $\text{CT}_{\text{max}}$  should be estimated using more realistic heating rates that have greater

**TABLE 1** Considerations for making critical thermal maximum (CT<sub>max</sub>) research more ecologically relevant

Points of interest	Issues with the current situation	Recommendations to make CT <sub>max</sub> more ecologically relevant
Thermal ramping	<ul style="list-style-type: none"> <li>Lack of consistent thermal ramping across studies</li> <li>Ramping rate varies during experiments</li> <li>Thermal ramping rate sometimes not reported</li> <li>Chronic rates are valid in many contexts but should not use the term CT<sub>max</sub> to describe endpoints</li> </ul>	<ul style="list-style-type: none"> <li>Disclosure and validation of rate of thermal ramping</li> <li>Rate of thermal ramping must be acute and ramped at a consistent rate until loss of equilibrium (LOE) is observed</li> <li>Highlight more cases of acute thermal ramping in natural environments and study these species</li> </ul>
Acclimation	<ul style="list-style-type: none"> <li>Fish are not always acclimated long enough</li> <li>Details of acclimation are not always disclosed</li> <li>CT<sub>max</sub> is not comparable due to choice of acclimation temperature</li> </ul>	<ul style="list-style-type: none"> <li>If attempting to compare CT<sub>max</sub> endpoints to other studies for predictive purposes, ensure fish are fully acclimated to high temperatures using measurable indices.</li> <li>Acclimate fish to different temperatures to determine the degree of phenotypic plasticity</li> <li>Report the duration of acclimation</li> </ul>
Measures of repeatability and heritability	<ul style="list-style-type: none"> <li>Laboratory-derived estimates do not represent those that would be derived in the wild</li> <li>No evidence for evolutionary rescue</li> </ul>	<ul style="list-style-type: none"> <li>Perform field CT<sub>max</sub> assays on wild fish to determine whether CT<sub>max</sub> is repeatable under natural conditions</li> <li>Develop a greater understanding of underlying mechanisms involved in LOE. Use experimental evolution and artificial selection to test adaptation potential in diverse species</li> </ul>
Thermal performance	<ul style="list-style-type: none"> <li>Few links between CT<sub>max</sub> and performance indices</li> <li>Some frameworks (aerobic scope and CT<sub>swim</sub>) are more useful to measure functional performance rather than CT<sub>max</sub></li> </ul>	<ul style="list-style-type: none"> <li>Continue exploring the possibility of having a “thermal type” and how these relate to performance traits</li> <li>Identify correlations between CT<sub>max</sub> and alternative indices of thermal tolerance and performance</li> </ul>

ecological relevance (e.g., Bartlett *et al.*, 2022; Mora and Maya 2006; Vinagre *et al.*, 2015). Very slow rates of warming (weeks to months) may be more representative of natural thermal challenges in some environments, and thus are more likely to shape responses of fishes to warming climates (Bates & Morley, 2020; Vinagre *et al.*, 2015). Yet, physiological responses to slower or chronic rates of thermal ramping have been found to vary, with some studies claiming that acclimation occurring during trials leads to overestimation of CT<sub>max</sub> (Beitinger *et al.*, 2000; Elliott & Elliott, 1995). Others suggest that prolonged exposure to higher temperatures leads to cumulative thermal stress and lower thermal tolerance (Rezende *et al.*, 2014; Terblanche *et al.*, 2007).

A recent study by Åsheim *et al.* (2020) demonstrated a positive correlation between rapid (0.3°C min<sup>-1</sup>) warming tolerance and slow (12-h heating) warming tolerance in lab-reared zebrafish, indicating that similar processes can govern thermal tolerance under both rapid and slow warming. Nonetheless, growth rates at high temperatures failed to correlate between the rapid and slow (12-h heating) warming groups. This suggests that chronic responses to thermal stress are likely governed by different physiological processes than acute warming tolerance, because chronic responses often involve decreased growth rates and cessation of feeding (Åsheim *et al.* 2020). A few other recent studies investigating the relationship between acute and chronic methods failed to identify relationships between the two, including in Atlantic salmon (*Salmo salar*; Bartlett *et al.*, 2022) and Atlantic cod (*Gadus morhua*; Zanuzzo *et al.*, 2019). Given these contrasting findings, it is difficult to determine whether slow and acute rates describe the same processes involved in thermal tolerance in wild fishes. Both chronic and acute warming

tolerance tests provide complementary views on how organisms respond to warming, but through different physiological mechanisms. Both views can provide valuable insight for how selection might occur in response to climate change, depending on the context or even the species (Åsheim *et al.* 2020; Bartlett *et al.*, 2022).

Acclimation rates vary among species (Chung, 2001; Lutterschmidt & Hutchison, 1997; Vinagre *et al.*, 2015), whereas lag time (*i.e.*, time for the body temperature to reflect the water temperature) depends on circulation of oxygen to tissues, as well as the surface area-to-volume ratio of the fish. Both acclimation rates and lag time are species-specific mechanisms that may also vary with ontogeny (Stevens & Fry, 1974). For these reasons, the effects of thermal ramping rates on CT<sub>max</sub> and, consequently, the ecological relevance of these rates, can vary among species. Nonetheless, slower (degrees per day) or chronic heating (weeks or months) rates ultimately measure different aspects of thermal tolerance, because CT<sub>max</sub> specifically refers to a response derived from an *acute* thermal stress challenge (Lutterschmidt & Hutchison, 1997). Using the term CT<sub>max</sub> to describe thermal tolerance derived using slow/chronic rates of thermal ramping adds variation to CT<sub>max</sub> values reported in the literature and hinders the detection of patterns and efforts to use CT<sub>max</sub> within an ecological context. It is, therefore, essential to accurately measure, report and justify the methodological details of the study when interpreting the data and the results.

Although it is important to acknowledge that rapid rates of thermal ramping may rarely occur in the wild, survival during short-duration heat shock (from minutes to hours) or heat waves (hours to days) can also be important in determining thermal limits (Box 2; see

## BOX 2 Case study on a population of *Oncorhynchus mykiss* living close to their upper thermal limit in Southern California

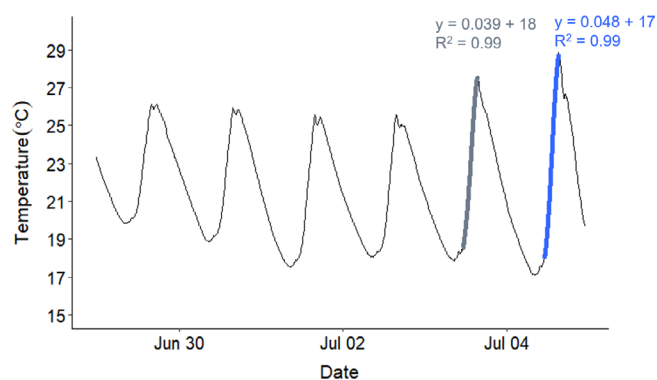
In Southern California, many streams and rivers are characterized as “intermittent,” meaning that they dry out in the summer, and aquatic organisms are confined to isolated refuge pools for several months before flows resume (Bogan *et al.*, 2019). During periods of drought, stream intermittency is even more widespread, and refuge pools are prone to becoming degraded or drying out entirely (Vander Vorste *et al.*, 2020). In these conditions, organisms are more likely to be exposed to rapidly increasing temperatures approaching their upper thermal limits. Thus, critical thermal maximum ( $CT_{max}$ ) tests with rapid thermal ramping may be more ecologically relevant for species inhabiting these systems. In this case study, the authors deployed environmental data loggers in a stream that experienced extreme drying during the summer of 2021 to assess whether wild *Oncorhynchus mykiss* in Southern California streams experience temperatures that approach their  $CT_{max}$  (c. 24–31°C depending on habitat temperature and heating rate; McKenzie *et al.*, 2020) and, if so, what is the rate of ramping to these temperatures?

In June 2021, *O. mykiss* were observed in several isolated pools in Piedra Blanca Creek (Ventura County, CA, USA). In one drying pool measured at 28°C, *O. mykiss* were observed dead or rapidly ventilating, confirming that 28°C can be lethal for these fish. To capture diel temperature changes during drying, a data logger was deployed upstream in a pool that was recently cut off from stream flow (Photo 1) and where *O. mykiss* were observed behaving normally. When the pool dried to c. 30 cm of water depth in July 2021, temperatures reached 28°C and 29°C during the day (Box Figure 1), once again confirming that environmental temperature can approach  $CT_{max}$  for these fish. A regression analysis revealed that the rate of heating during these last 2 days before the logger dried out measured 0.039 and 0.048°C min<sup>-1</sup>, respectively, well below the standard 0.3°C min<sup>-1</sup> (Box Figure 1).

The authors conclude that environmental temperatures can, in fact, approach  $CT_{max}$  for *O. mykiss* inhabiting intermittent streams in Southern California but that rates of temperature increase are far lower than the typical rate for a  $CT_{max}$  test. These fish can face repeated and ultimately lethal ramping to  $CT_{max}$  temperatures during summer heat waves and risk extirpation if winter rains do not sufficiently re-hydrate their habitat.



**PHOTO 1** An isolated pool containing *O. mykiss* where a data logger was installed to monitor temperature



**BOX FIGURE 1** Temperature data from the submersible miniDOT logger (Precision Measurement Engineering, Vista, CA, USA) for the final 5 days before the logger went dry (pool depth would have been c. 30 cm when the logger was dry). Regression lines and corresponding equations represent the temperature ramping rates for the final 2 days



Åsheim *et al.* 2020). Fish can experience rates similar to those used in  $CT_{max}$  protocols under certain conditions, such as in the intertidal zone, during extreme upwelling events or when moving through a thermocline (Bates & Morley, 2020; Genin *et al.* 2020). Although fast rates of heating often overestimate functional thermal tolerance (Becker & Genoway, 1979), evidence suggests that  $CT_{max}$  estimates are closely related to global distribution of fish species (Payne *et al.*, 2021; Sunday *et al.*, 2012). Mass mortality events of ectotherms have also been caused by acute thermal shock in the wild (e.g., Finnegan *et al.*, 2012; Genin *et al.* 2020; Penn *et al.*, 2018; Vertessy *et al.*, 2019; Wegner *et al.* 2008).  $CT_{max}$  can, therefore, be a useful tool to determine responses to these thermal events in the future. The rate of change in the temperature regime itself may, in fact, be more influential than experimentally derived endpoints when predicting survival in fish, because the stress response induced during acute thermal ramping increases pathogen-related mortality (Alfonso *et al.*, 2021; Genin *et al.* 2020). As such, the increased prevalence of heat waves predicted to occur in the near future (Frölicher *et al.* 2018; Allan *et al.*, 2021) can either act as a force driving directional selection or exemplify the concept of “plastic rescue,” where individuals are able to reach higher limits due to previous exposure to thermal stress.

In summary, rapid rates of thermal ramping may not always represent conditions in the wild, but upper thermal limits obtained from this approach are still useful. Inconsistent rates of ramping across different studies will lead to overestimation or underestimation of critical thermal limits, which is why  $CT_{max}$  estimates must be interpreted in the context of the animal's thermal history, as well as in the experimental design and protocol that generated the estimate. Given that  $CT_{max}$  is characterized by acute responses to thermal challenges, researchers should proceed with caution when using  $CT_{max}$  to describe estimates obtained using thermal ramping rates that occurred over longer time scales. These estimates may appear to be more ecologically relevant but are underpinned by fundamentally different mechanisms that limit thermal tolerance (such as protein denaturation vs. oxygen or energy limitations; Brandts, 1964; Jutfelt *et al.*, 2021).

## 2.2 | How does acclimation influence $CT_{max}$ ?

Studies attempting to determine thermal limits often encounter difficulty in making predictions owing to the effects of acclimation. Acclimation occurs when animals reach a new stable state in rate processes after being exposed to a period of thermal adjustments (Seebacher *et al.*, 2015), typically achieved over 4–5 weeks (Johansen *et al.*, 2021; Schulte *et al.*, 2011). Researchers can establish whether organisms have been successfully acclimated by measuring metabolic rates, in particular, biomarkers, such as red muscle citrate synthase and lactate dehydrogenase activities, blood glucose and haemoglobin concentrations, spleen somatic index and gill lamellar perimeter and width (Johansen *et al.*, 2021). Nonetheless, it is important to note that thermal compensation from previous acute thermal exposure may

influence standard metabolic rate and may lead researchers to assume a fish is fully acclimated when it may not be (Evans, 1990).

Although it is widely accepted that acclimation influences upper thermal tolerance in fish (Beitinger & Bennett, 2000; Huey *et al.*, 2012; Schulte *et al.*, 2011), the underlying physiological mechanisms remain poorly understood (Ern *et al.*, 2016; Lefevre *et al.*, 2021; McKenzie *et al.*, 2020), and individual, population and species-level differences can have confounding effects. Discrepancies in acclimation (*i.e.*, presence, absence or lack of reporting) have important ramifications on the measured  $CT_{max}$  values, making it difficult to compare the results across studies or perform meta-analyses or data syntheses with existing literature (Lutterschmidt & Hutchison, 1997).

Generally, acclimation effects in fish can be observed across a large range of temperatures. Acclimation to higher temperatures typically yields higher  $CT_{max}$  values, with values converging towards an asymptote as temperature increases (Chen *et al.* 2015; McKenzie *et al.*, 2020; Morgan *et al.*, 2019). In wild zebrafish (*Danio reiro*), individuals living in warmer habitats had higher  $CT_{max}$ , likely due to acclimation (Morgan *et al.*, 2019). In addition, the term “acclimation” is frequently misused to refer to the relatively short adjustment period (also referred to as habituation) between the introduction of the organism into the  $CT_{max}$  apparatus and the start of the trial (Bates & Morley, 2020). Similarly, very few  $CT_{max}$  studies actually measure any indices of acclimation. Recording measurable changes in energy expenditure from one stable state to the next (*i.e.*, from one temperature to another) might require experimental trials to last several months, rather than a few weeks, depending on the magnitude and rate of environmental change (Beitinger & Lutterschmidt, 2011). Although it may not always be possible to test whether acclimation was achieved during experimental trials, it is particularly important to disclose the details of the adjustment period prior to experimental trials, to generate repeatable or comparable results (Beitinger & Lutterschmidt, 2011). The rate at which fish can adjust to changing conditions may in part determine which species will survive under future climate scenarios (although mobile species may be able to relocate to suitable habitat conditions elsewhere). Fish with a capacity for rapid acclimation, provided energetic reserves are not depleted, may also cope better with climate change (Somero, 2010). In fact, adaptation can be accelerated by plasticity (Chevin & Lande, 2010; Lande 2009; West-Eberhard 2003), which indicates that there is some positive genetic correlation between acclimation phenotypic plasticity and  $CT_{max}$ . Morgan *et al.* (2020) quantified the contribution of acclimation to upper thermal tolerance over six generations of artificial selection to higher thermal tolerance in zebrafish and found that the acclimation capacity declined when the populations evolved higher thermal tolerance. Furthermore, adaptation lagged behind the current rate of warming. These findings suggest that there may be low potential for evolutionary rescue in tropical populations of fish that already live close to their thermal extremes. The effects of acclimation may provide greater benefit in populations living in temperate environments where seasonal fluctuations in temperature are more predictable (Morley *et al.*, 2019; Nati *et al.*, 2021; Rummer *et al.*, 2014; Ryu *et al.*, 2020; Wang & Dillon, 2014), although previous studies failed to

find a link between plasticity and latitude or seasonality (Gunderson & Stillman, 2015).

Future studies attempting to predict responses to climate change should focus on determining acclimation potential in wild populations. Pushing acclimation towards higher temperatures when performing  $CT_{max}$  assays will reduce the variability in estimated thermal limits (especially in temperate species) and provide a more accurate prediction of temperature extremes at which fish can survive. Determining rates of acclimation over a range of temperatures in populations of different species will facilitate comparisons of populations living in different thermal regimes and also between temperate and tropical species (e.g., Morley *et al.*, 2019). To increase the accuracy of  $CT_{max}$  estimates for predicting future species distributions, fish should be fully acclimated prior to conducting  $CT_{max}$  trials, and this acclimation should be confirmed using reliable measurable indices (e.g., metabolic rate). Finally, the rate of acclimation should always be reported, as it is important to understand how fish will survive periods of exposure to supra-optimal temperatures beyond the context of acute warming.

### 2.3 | How does $CT_{max}$ compare to other estimates of thermal performance?

Efforts to understand the extent to which  $CT_{max}$  relates to organismal performance are needed to assess the ecological relevance of the metric. For example, questions such as whether fish with higher  $CT_{max}$  swim better in supra-optimal conditions or whether fish with lower  $CT_{max}$  are less likely to forage in warmer waters can be explored. If it can be linked to either increased or decreased performance,  $CT_{max}$  can be used as a proxy for thermal performance during heat waves or in areas with warm-water discharge. The development and testing of conceptual frameworks that attempt to link  $CT_{max}$  to performance traits will help to predict responses to climate change, as well as explore the physiological responses of organs involved in the response to thermal stress.

Several studies have used thermal performance curves (TPC) as a tool to determine how different species respond to climate change (Dillon *et al.*, 2010; Deutsch *et al.* 2008; Huey *et al.*, 2012; Sinclair *et al.*, 2016). TPCs describe the relationship between body temperature and performance in ectotherms. These curves are fundamentally characterized by low performance at critical thermal limits (minimum and maximum), maximal performance at an optimal temperature and a temperature range at which performance remains above 50% of its maximum (Rezende & Bozinovic, 2019). Performance indices include behaviour, life-history traits and physiological variables in ectothermic organisms (Rezende & Bozinovic, 2019). At the whole-organism level, performance traits of interest often include fecundity, growth, metabolic rate and swimming speed (Schulte *et al.*, 2011). At tissue and cellular scales, performance traits may include heart rate, nerve conduction velocity, mitochondrial function and enzyme activity. Metrics of performance typically include biological rate processes, such as offspring per lifetime, amount of oxygen consumed per unit time, distance travelled per unit time and enzyme reaction rates (Schulte

*et al.*, 2011). The increase in performance as temperatures reach optimal levels is thought to reflect fundamental effects of thermal dynamics on molecular movements, whereas the decrease at supra-optimal temperatures is linked to temperature-dependent destabilizing effects, including reversible or irreversible protein denaturation (Schulte, 2015; Schulte *et al.*, 2011). The shape and breadth of TPCs can vary across levels of biological organization, as well as within and between species, based on seasonal patterns, such as reproduction or migration, with phenotypic plasticity, geographic location and time (Eliason *et al.*, 2011; Rezende & Bozinovic, 2019; Schulte *et al.*, 2011). Even so, greater understanding of mechanisms underlying the responses of organisms to thermal stress and how TPCs translate to the success of fish in nature is incomplete yet fundamental for improving the interpretation of differences in the shape of TPCs (Rezende & Bozinovic, 2019; Schulte *et al.*, 2011).

Because  $CT_{max}$  is measured using acute thermal ramping, TPCs generated under similar rapid rates of heating provide insight into how  $CT_{max}$  relates to the trait being measured (e.g., Dowd *et al.*, 2015; Kingsolver & Woods, 2016; Rezende *et al.*, 2014). For example,  $CT_{max}$  can be related to short-term performance traits by conducting an experiment during which fish are forced to swim while exposed to increasing temperatures until a fish experiences the fatigue that occurs prior to LOE (sometimes referred to as  $CT_{swim}$ ). This type of experiment would help researchers directly relate  $CT_{max}$  to swimming speeds and provide clear insight on how acute thermal stress impacts performance.

Previous studies have attempted to measure swimming performance in relation to temperature increases as an alternative to the classical  $CT_{max}$  endpoint, though they have measured different endpoints. Steinhausen *et al.* (2008) measured  $T_{crit}$ , the point at which aerobic scope equals zero, during swim trials where temperature was increased every 30 min. Although  $T_{crit}$  fails to account for the switch from aerobic metabolism to anaerobic metabolism, additional steps to measure an endpoint that considers this transition during the swimming challenges would facilitate comparisons to  $CT_{max}$ . Blasco *et al.* (2020) investigated whether  $CT_{swim}$  (the temperature at which fish cease to swim when progressively warmed) could be used as an alternative to LOE in  $CT_{max}$  experiments. Although they attempted to relate  $CT_{swim}$  to a form of  $CT_{max}$ , Steinhausen *et al.* (2008) and Blasco *et al.* (2020) used a slow rate of ramping (1°C per 30 min) which deviates from the standardized procedure. Relating  $T_{crit}$  or  $CT_{swim}$  measurements to  $CT_{max}$  measurements on the same individuals using an acute rate of ramping would provide insight into how swimming activity relates to  $CT_{max}$ .

In some instances, measuring LOE may not be feasible, thus requiring researchers to modify the suggested CTM protocol. For instance, morphological or physiological differences in study organisms can alter the LOE response and lead to over- or underestimated  $CT_{max}$  values. In lumpfish (*Cyclopterus lumpus*), for example, it can be particularly difficult to measure LOE because they have a suction disc that they use to attach strongly to rocks or other surfaces. For benthic fish, or those without a swim bladder, other endpoints include spiracle cessation (stingrays) or onset of muscle spasms (Bouyoucos

*et al.*, 2020) or loss of righting response (experimenter disorients fish with probe and waits for re-righting, Andreassen *et al.* 2022; Fangué and Bennett 2003). Understanding how LOE relates to alternative sublethal endpoints would, therefore, provide a greater understanding of physiology involved in LOE while expanding the CTM to include a set of measurable, well-justified alternative endpoints. It is important to note that these unconventional endpoints will likely be species- or context-dependent. They may be particularly useful when extrapolating results to the wild, especially considering that LOE rarely occurs and often means ecological death in natural settings (*i.e.*, animals cannot avoid predation or seek cooler refuges). Nonetheless, if alternative endpoints are selected, researchers should opt to maintain other key characteristics of CTM, including acute rates of ramping and high post-trial survival rates.

Some studies have opted to conduct thermal performance experiments over longer time scales to mimic how the degree of thermal stress experienced under prolonged exposure regimes influences key animal response (*e.g.*, reproduction; Deutsch *et al.* 2008). Indeed, cumulative effects of sublethal and long-term temperatures may influence energy balance (Dillon *et al.*, 2010), fecundity and developmental rates (Huey & Berrigan, 2001) and ultimately fitness (Rezende & Bozinovic, 2019). Upper thermal limits for physiological performance traits (*e.g.*, aerobic scope and cardiac scope) differ from  $CT_{max}$ . Nonetheless,  $CT_{max}$  remains useful as an index for comparison against these upper thermal limits for performance traits and can be applied at both the species and the individual levels.

There is also the possibility that  $CT_{max}$  relates to functional traits derived under slower rates of heating that are more commonly observed in the wild (degrees per day), because these indices may share similar underlying mechanisms (Åsheim *et al.* 2020). Some ectotherms display a thermal syndrome or “thermal type,” where some individuals are consistently cold-tolerant and others consistently warm-tolerant (Goulet, Thompson, & Chapple, 2017). The notion of “types” is based on a theoretical framework for studying correlated traits (at both inter- or intraspecific levels), and it takes into consideration the links among temperature, metabolism and behaviour. Goulet *et al.* (2017) suggested that an individual's thermal type would align with behavioural and life-history types. Cold-type individuals would have a cold-shifted TPC, whereas warm-type individuals would have a warm-shifted TPC. As previously mentioned, Åsheim *et al.* (2020) observed a correlation between thermal tolerances obtained from rapid and slow rates of warming. Thus, there were individuals with consistently (relatively) higher thermal tolerance, acting as a “warm-type,” and others with consistently (relatively) lower thermal tolerance. The study also reported a lack of correlation between thermal tolerance derived under rapid heating and growth at a higher than optimal temperature, suggesting that acute thermal tolerance has little mechanistic association with growth performance under supra-optimal temperatures. This observation may reflect a very limited scope for a thermal syndrome (*e.g.*, warm-type individuals performing better than cold-type conspecifics). Nonetheless, more research would be required to determine whether alternative mechanisms of

thermal tolerance can be organized into some form of thermal syndrome, as found in reptiles (Åsheim *et al.* 2020; Goulet *et al.* 2017; Goulet, Thompson, Michelangeli 2017; Michelangeli *et al.*, 2018).

$CT_{max}$  has been selected to compare thermal performance across individuals in the field (Desforges *et al.*, 2021). Desforges *et al.* (2021) attempted to link  $CT_{max}$  to traits such as growth, migration strategy and predation vulnerability, but they found no evidence that differences in  $CT_{max}$  were associated with variation in these traits. By contrast, studies that used alternative indices of tolerance to warming, such as cardiorespiratory performance traits in different sockeye salmon populations, have identified links with performance traits related to migration difficulty (*i.e.*, distance and effort required to reach spawning grounds) and temperatures experienced in the past (Eliason *et al.*, 2011). In contrast, Chen *et al.* (2013) measured  $CT_{max}$  in laboratory-reared juvenile sockeye salmon from the populations outlined in Eliason *et al.* (2011) and found  $CT_{max}$  to be higher in populations with greater migration difficulty. This suggests that the physiological mechanisms underlying  $CT_{max}$  have ecologically relevant applications, because they are linked to endurance and ability to cope with strenuous challenges.

Regardless of the index used to estimate upper thermal tolerance, each type of thermal performance measured comes with limitations; the physiological mechanisms underlying these responses are complex and may not be fully described by a single measure (Lefevre *et al.*, 2021; Rezende *et al.*, 2014; Rezende & Bozinovic, 2019). The degree of uncertainty associated with the physiological mechanisms involved in LOE is a major limitation to the applicability of  $CT_{max}$ . There is some speculation that vital organs, such as the brain or heart, are responsible for performance decline during acute thermal stress (Lefevre *et al.*, 2021). Nonetheless, CTM does not provide a way to assess the impacts of longer exposures to sublethal temperatures on other organs (Lefevre *et al.*, 2021). Organs can fail at similar temperatures, but across different durations of exposure (Lefevre *et al.*, 2021). Some studies advocate for the use of an ecologically relevant sublethal threshold based on fatigue from exercise performance as an endpoint rather than LOE. Blasco *et al.* (2020) argued that  $CT_{swim}$  provides a more ecologically relevant sublethal threshold for tolerance of acute warming than LOE in fishes. Nonetheless, like  $CT_{max}$ , the mechanisms that lead to fatigue in  $CT_{swim}$  tests are not fully understood, although they may be similar across species and, therefore, easier to investigate (Blasco *et al.*, 2020). Although TPCs are also useful methods to gain insight on thermal limits, they provide more information on how specific physiological systems react to thermal challenges.

## 2.4 | What are thermal safety margins, and how can $CT_{max}$ be used to determine them?

Thermal safety margins generally refer to an excess of upper thermal tolerance (*e.g.*, Deutsch *et al.* 2008; Huey *et al.*, 2012; Sunday *et al.*, 2014) and can be used to predict and compare the sensitivity of a particular species to thermal stress (*e.g.*, Pinsky *et al.*, 2019). Several



approaches have been developed to quantify thermal safety margins. Although most have used  $CT_{max}$  as a proxy for upper thermal tolerance, the environmental parameter used to determine the width of this thermal margin often varies. Some examples include acclimation temperature (McArley *et al.*, 2017; McKenzie *et al.*, 2020), highest hourly body temperature in the coolest microhabitat available (Pinsky *et al.*, 2019), maximum habitat temperature (Vinagre *et al.*, 2019) and highest mean monthly temperature (Comte & Olden, 2017), all used as metrics to derive thermal safety margins. Given the many ways of defining thermal safety margins, it is important to explain and justify the selected method to describe sensitivity.

Given that  $CT_{max}$  is influenced by acclimation temperatures, upper boundaries (and thus thermal safety margins) can be somewhat flexible – especially in fish species not living near their thermal extremes. Species with broad geographic ranges may exhibit different levels of phenotypic plasticity and  $CT_{max}$  owing to population-level adaptation to local environmental conditions (Comte & Olden, 2017). Attempts have been made to account for this variability while modelling species distribution and predicting responses to climate change (*e.g.*, Comte & Olden, 2017; Pinsky *et al.*, 2019; Sunday *et al.*, 2014). Thus, it is important to account for plasticity in thermal responses and thermal history when calculating  $CT_{max}$  values across different species or in a single species with a large geographic range (see Comte & Olden, 2017).

Thermal safety margins can also be overestimated (*i.e.*, too broad) if the experimental data used were compiled with arbitrary acclimation temperatures (*i.e.*, temperatures that are not ecologically relevant but rather used for logistical purposes), which frequently occurs in  $CT_{max}$  studies (Sunday *et al.*, 2014). Tropical species experience relatively more stable annual temperature regimes (Frölicher *et al.*, 2018; Wang & Dillon, 2014) and are acclimated to higher temperatures. As such, tropical species have relatively narrow safety margins. Recently, Payne *et al.* (2021) found that tropical species actually show broader heating tolerances at a given acclimation temperature than temperate species, but narrower heating tolerances at higher temperatures. Although thermal safety margins appear greater in tropical species acclimated to the same optimal temperature as a temperate species, temperate species show greater capacity to cope with increases in temperature than tropical species do. Nonetheless, to make similar comparisons with temperate species easier,  $CT_{max}$  values would ideally be determined using the warmest temperatures these species experience in the wild, across their geographical range.

Methodological variation in  $CT_{max}$  protocols and subsequent over- or underestimation of  $CT_{max}$  can lead authors to make incorrect inferences on thermal safety margins. Incorrect predictions may also arise if  $CT_{max}$  values are not adjusted based on the highest acclimation temperatures experienced in the wild. Many studies that use CTM attempt to answer specific questions about a species or populations with the most appropriate methods for the particular study, without standardizing their results for inclusion in meta-analyses or data syntheses. To advance efforts in comparing interspecific upper thermal tolerance in the form of  $CT_{max}$ , it is essential to acknowledge the

importance of, and to report, methodological differences in CTM particularly when estimating thermal safety margins and modelling responses to warming.

## 2.5 | Is $CT_{max}$ repeatable, and what does that tell us about heritability and adaptive potential?

In ecological and evolutionary research, repeatability of response traits tends to be positively correlated with heritability (Boake 1989; Bell *et al.*, 2009; Dohm, 2002). In fact, Boake (1989) suggested that repeated measures allow researchers to make inferences about rates of evolution, because both the rate of evolution and the magnitude of heritability are constrained by repeatability. Thus, the repeatability of thermal tolerance estimates has been used as an approach to make inferences about the adaptive potential of species in relation to climate change or other environmental changes (Killen *et al.*, 2016; Morgan *et al.*, 2018). To assess the adaptive capacity of a population, there must be phenotypic variation in the trait of interest, the trait must be heritable and there must be selection for the trait. Here, heritability is defined as the ratio between the amount of additive genetic variance and the amount of phenotypic variance of a specific trait within a population (Falconer & Mackay 1996). A heritability value close to one implies that almost all of the variability in a trait comes from genetic differences, with very little contribution from environmental factors. Repeatability shows the consistency of an individual's performance over time, by quantifying the proportion of total variation of a trait that is due to differences between individuals (Bell *et al.*, 2009; Dohm, 2002). Within-individual repeatability refers to the degree of consistency in reproducing a trait of interest over time in an individual subject (*i.e.*, temporal stability of a trait), whereas between-individual repeatability accounts for the proportion of total variation for a trait within a population (Killen *et al.*, 2016).

The potential for evolutionary responses under a warming climate may be estimated by the repeatability of individual  $CT_{max}$  in a variety of species and populations, over both short and long time scales, across life stages and under a range of ecologically relevant environmental conditions. High repeatability of  $CT_{max}$  would suggest that the trait is, at least partially, controlled by genetic variation, thus providing a mechanism upon which natural selection can occur. Repeatable traits are temporally stable to be subject to selection and are thus likely to evolve. For example, fish with consistently low  $CT_{max}$  under a wide range of environmental conditions might be more susceptible to heat waves than conspecifics with higher  $CT_{max}$ . Researchers have only recently started to explore how repeatable  $CT_{max}$  estimates are for individual organisms and under what conditions.

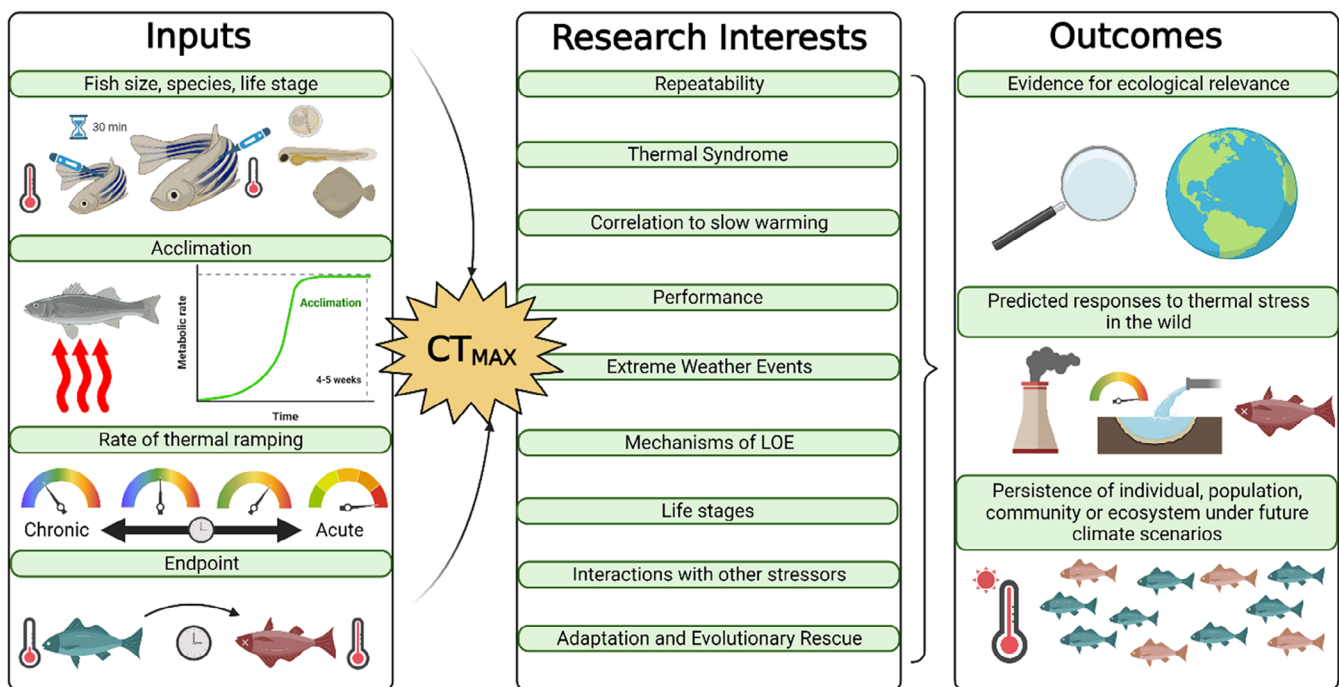
Repeatability of  $CT_{max}$  estimates would support its ecological relevance. Indeed, several studies provide support for  $CT_{max}$  being a repeatable trait within individuals of diverse species. Morgan *et al.* (2018) investigated the repeatability of  $CT_{max}$  in zebrafish (*Danio rerio*) and found the repeatability coefficient to be 0.45 (on a scale of 0 to 1, where values closer to 1 represent greater repeatability). The

findings of Morgan *et al.* (2018) revealed that although  $CT_{max}$  seems to be repeatable, it is unclear how much of the repeatability can be associated with environmental history and how much can be associated with genetics. The genetic variability underlying thermal tolerance provides a basis for natural selection to occur, allowing populations to evolve or alter their thermal tolerance. This phenomenon can have important benefits for range expansion or species redistribution and improved coping with global climate change (Morgan *et al.*, 2018). In addition to short time scales (days to weeks), others have found evidence of repeatability in fish over longer time scales. For example, O'Donnell *et al.* (2020) reported a repeatability coefficient of 0.48 for trial 1 month apart to 1 year apart in brook trout (*Salvelinus fontinalis*), and Grinder *et al.* (2020) reported a coefficient of 0.43 for 6 weeks in the Trinidadian guppy (*Poecilia reticulata*). Other studies that used alternative methods to measure upper thermal tolerance in fishes have also found evidence of heritability (e.g., Anttila *et al.* 2013; Munoz *et al.* 2014; Perry *et al.* 2005), suggesting that thermal tolerance may be (at least partly) heritable, whether it be estimated using  $CT_{max}$  or other methods.

Although acute upper thermal tolerance likely has a genetic component, stronger evidence of relationships between repeatability and heritability in  $CT_{max}$  under natural conditions is still lacking. Because heritability is influenced by phenotypic variability, heritability can decrease under natural conditions owing to increased individual phenotypic plasticity (Dingemans *et al.*, 2010; Killen *et al.*, 2016; Nussey

*et al.*, 2007). A meta-analysis by Bell *et al.* (2009) found that several behavioural traits that were repeatable often differed among age classes, across sexes (also reported in O'Donnell *et al.* 2020) and between field and laboratory studies. Many factors can elicit plasticity (variation) in  $CT_{max}$  estimates, including differences in life stages (e.g., Illing *et al.*, 2020; Recsetar *et al.*, 2012), diet (Isaza *et al.*, 2019), water quality (e.g., Ern *et al.*, 2016; Potts, 2020; Sardella *et al.*, 2008), habitat (Rodgers *et al.*, 2019), reproductive stage (Auer *et al.*, 2021; Dahlke *et al.* 2020; Johnson, 1976; Wheeler *et al.*, 2022) and social status (Gilmour & Bard, 2022; LeBlanc *et al.*, 2011). Chronic stress in fish can also impair responses to thermal stress (e.g., Gilmour & Bard, 2022; Claireaux *et al.*, 2013; LeBlanc *et al.*, 2011). These observations raise the question of whether  $CT_{max}$  is as repeatable within individuals in the wild and, therefore, potentially heritable across generations, as it appears to be under controlled laboratory conditions, particularly because environmental effects could mask genetic differences (Bell *et al.*, 2009).

Repeatability is, therefore, context-dependent, and under particular conditions, a trait with high repeatability, like thermal tolerance, can effectively impact ecological performance and fitness (Claireaux *et al.*, 2013; McKenzie *et al.*, 2020). In mesocosm experiments, Claireaux *et al.* (2013) exposed European seabass (*Dicentrarchus labrax*) to stressful conditions (oil or chemically dispersed oil) and found evidence of repeatability in thermal responses. A control group, where fish were not exposed to oil or chemical dispersant, revealed



**FIGURE 1** Conceptual diagram outlining the links between methodological inputs for estimating critical thermal maximum ( $CT_{max}$ ), research interests and potential outcomes.  $CT_{max}$  should be measured by considering important inputs, such as fish size, acclimation, rate of thermal ramping and an endpoint. Using an index of upper thermal tolerance derived while considering these inputs, studies can address questions that relate to progressive concepts, such as repeatability, thermal syndrome, correlation to slow warming, performance, extreme weather events and mechanisms of loss of equilibrium (interests). In turn, these studies can be used as evidence of ecological relevance and applied to predict responses to thermal stress under climate change scenarios (outcomes)

**BOX 3 Research needs.** The authors identified research priorities by using a word cloud software to extract the top 50 words from each of the five considerations discussed in the previous sections. They then generated a subsequent word cloud using these extracted words to narrow down the 10 most common terms discussed. This method yielded the following list of words: climate, estimates, stress, conditions, change, acute, rate, time, species and fitness. Acute, time and rate are related terms and thus discussed as one theme. The authors opted to add the term context, as it is central to research in the field of thermal biology. As such, they present the following list of nine themes to help focus research aimed at optimizing the use of critical thermal maximum ( $CT_{max}$ ) in the context of ecology

### Research needs

#### Climate

Understanding how  $CT_{max}$  relates to historical, present and predicted climate scenarios will provide insight on how individuals, populations and species will respond to temperature fluctuations and extreme weather events. Identifying patterns in  $CT_{max}$  estimates that coincide with historical extreme weather events, whether observed at local or regional scales, within-populations or across species, will further highlight the relationship between  $CT_{max}$  and survival, a proxy for Darwinian fitness.

#### Estimates

As with any metric of thermal tolerance, there is uncertainty associated with the underlying mechanisms of loss of equilibrium (LOE), which is why they are considered estimates (with some uncertainty) of upper thermal limits. Reducing this uncertainty – either by standardizing protocols or integrating  $CT_{max}$  with functional metrics – will improve accuracy in forecasting responses to warming.

#### Thermal stress

The physiological and biochemical pathways that modulate thermal stress responses at different time scales (resistance, acclimation, adaptation) are not fully understood. Investigating how thermal stress manifests itself across levels of biological organization (cellular to whole-organism) will assist in linking  $CT_{max}$  to performance and fitness.

#### Conditions

Environmental conditions play an immense role in shaping thermal tolerance limits. Conducting  $CT_{max}$  trials in a field-based setting with wild fish can demonstrate more realistic links between this estimate of thermal tolerance and behavioural or physiological responses.

#### Change

Although current evidence suggests that evolutionary rescue might not be possible (Morgan *et al.*, 2019), further understanding the interplay between rates of environmental change and genetic change will be critical in assessing how warming will threaten different species. This is particularly important when considering  $CT_{max}$  estimates, as they can be heavily influenced by rates of thermal ramping.

#### Acute, time, and rate.

Future studies should acknowledge that  $CT_{max}$  measures responses to acute thermal stress and emphasize the importance of duration when conducting trials. The duration can physiologically and biochemically impact responses to thermal stress. Efforts should focus on determining the factors that underpin LOE to bridge the gap between acute and chronic thermal tolerance estimates.

#### Species

Fishes are incredibly diverse, and so responses to thermal stress may not be the same across species. As such, developing a systematic way of adjusting  $CT_{max}$  protocols to account for these differences would yield standardized results that could be used in meta-analyses and studies focused on interspecific differences.

#### Fitness

Fitness has been the ultimate focus of past and present studies on upper thermal tolerance, as researchers investigate performance traits such as swimming speed, aerobic scope, metabolic rates, fecundity and growth – all of which increase reproductive success and offspring survival when optimized. Understanding how these traits manifest themselves under acute thermal challenges will clarify the ecological relevance of  $CT_{max}$ .

#### Context

$CT_{max}$  methodologies change according to research questions and context. Accounting for factors variables as sex, population, and life stage (among others) is critical when considering species resilience.

repeatable measures of time to loss of equilibrium ( $T_{LOE}$ ), with a large degree of between-individual variation (Claireaux *et al.*, 2013). The authors used a different approach than  $CT_{max}$ , but their study still

provides insight into the relationship between repeatability and environmental stress. After a month of exposure to oil or a chemically dispersed oil, individuals that died earlier were found to have lower thermal

**BOX 4 Suggested series of considerations when performing critical thermal methodology**

## Considerations for CTM

- 1. Define research goals.** Critical thermal methodology (CTM) can be used in a variety of ways to answer a broad spectrum of research questions. Although it is acceptable to tailor CTM according to the context of the study, establishing and describing a thorough experimental design to address research goals will prevent researchers from ignoring the critical aspects of the CTM, such as fish size, origin, acclimation and rate of ramping.
- 2. Use the term critical thermal maximum (CT<sub>max</sub>) with care.** Researchers should use this term with caution. Although some variations in CTM are acceptable to meet the somewhat elusive standard, CTM should involve an acclimation period, an acute rate of ramping and loss of equilibrium (LOE) or a widely accepted alternative sublethal endpoint. Chronic rates of ramping, lethal endpoints and use of performance indices rather than sublethal endpoints are not considered CTM, though they can be useful techniques to use in combination with CTM or independently, depending on the context.
- 3. Establish the size range of study organisms.** Larger fish will experience temperature lags, which could influence the endpoint and subsequent conclusions. The ideal experimental design would ensure fish are relatively uniform in size when logistically possible and discuss analyses performed to account for size differences. It is recommended to conduct trials on select individuals of varying sizes that measure the internal temperature of the fish using a probe prior to beginning CT<sub>max</sub> experiments. This will allow researchers to determine the magnitude of the temperature lag (if there is any) and control for this effect during subsequent analyses.
- 4. Acclimate fish.** Although this varies according to research contexts, a decision should be made about how the term “acclimation” will be used during the study. Selecting a temperature at which the fish maintain a stable physiological state for an extended period of time is the typical procedure. Additional steps can be taken to quantify acclimation by measuring metabolic rates, though recent thermal history and acute temperature exposure should be accounted for when assessing whether a fish is fully acclimated.
- 5. Select rate of ramping.** CTM involves steady, acute rates of thermal ramping. Thermal ramping that occurs over several days to weeks or longer derives a metric of chronic thermal tolerance, not CT<sub>max</sub>. The selected rate of ramping should be fast enough to induce acute thermal stress responses (rapid opercular movements, erratic swimming behaviour and eventually LOE) but slow enough to reduce temperature lags in the larger fish.
- 6. Tailor endpoints to the organism and context.** CTM typically involves the use of LOE as an endpoint. Although alternative sublethal endpoints can be used to evaluate acute upper thermal tolerance in species with unique morphological features such as rays or flatfish, a thorough justification should be provided to support the selected endpoint, especially if referring to this metric as CT<sub>max</sub>. In addition, it is critical to discuss the thermal history of the study organisms to account for the potential effects of previous exposure to thermal stress. Performing a literature search on previous acute thermal ramping challenges for potential study organisms (including life stage, sex, diet, size, etc.) could provide further insight on particular aspects of the experimental design that require modifications.
- 7. Measure additional indices.** When possible, researchers should aim to bridge the knowledge gap in understanding the underlying physiological mechanisms of LOE by measuring additional indices on a sub-set of experimental fish. Metabolic rate, swimming speed, acclimation capacity and genetics are examples of data that can further advance our understanding of acute upper thermal tolerance. Moreover, comparing CT<sub>maxima</sub> to chronic thermal tolerance estimates in individuals will further indicate the ecological relevance of CTM.
- 8. Be transparent about limitations.** Limitations that influence the use of derived metrics in future studies or the reproducibility of results should be accessible and discussed in detail in the manuscript.
- 9. Interpret data with caution.** CT<sub>max</sub> estimates for individuals can be compared to other individuals within the study, assuming they are all exposed to the same acclimation conditions and rates of ramping. Although the CT<sub>maxima</sub> value for all individuals within the study can be used to make inferences about population responses to acute thermal stress, methodological differences should be accounted for when comparing results to previous findings. Within the study, CT<sub>max</sub> can allow researchers to make predictions about genetic variation, responses to extreme weather events and climate-driven behavioural changes.

tolerance. In this case, tolerance to these thermal challenges predicted survival, a proxy for Darwinian fitness. The between-individual trait variation along with strong selective pressures led to a higher frequency of thermally tolerant individuals, promoting directional selection.

More research is required to better understand the links between repeatability and heritability, with particular consideration for differences between wild and laboratory conditions (Killen *et al.*, 2016). The potential for environmental factors and anthropogenic stressors to

shape  $CT_{max}$  should not be neglected when making inferences on the adaptive potential of populations to changing climate. Though several studies found high repeatability coefficients for  $CT_{max}$ , these results should be interpreted with caution because the degree to which environmental factors impact  $CT_{max}$  remains largely unknown. Repeatability often sets the upper limit to heritability (Dohm, 2002; Dochtermann *et al.*, 2015; Falconer, 1981; Killen *et al.*, 2016), and Morgan *et al.* (2018) found repeatability estimates to be greater than the heritability estimates from previous studies (*e.g.*, Baer & Travis 2000; Doyle *et al.* 2011). Nonetheless, when there are significant genotype-environment interactions, repeatability may not always set upper boundaries for heritability (Dohm, 2002).

Another important question is whether the rate of evolution of thermal tolerance is high enough to keep up with the rate of warming. By artificially selecting for  $CT_{max}$  over 6 generations of wild-caught zebrafish, Morgan *et al.* (2020) recently showed that although adaptation of upper thermal tolerance occurred, the rates of adaptation were slow. The study found evidence of both up-selection and down-selection of upper thermal tolerance, with up-selection being significantly slower ( $0.04 \pm 0.008^{\circ}\text{C}$ ) and reaching an upper limit (Morgan *et al.*, 2020). These findings imply that natural selection will be insufficient to generate rapid change, suggesting low potential for evolutionary rescue. More studies are needed to assess the potential for evolutionary rescue across diverse species, to identify mechanisms that may allow populations to adjust to new climate conditions and to determine how to re-enforce these mechanisms in conservation and management strategies as climate change escalates.

In summary, genetic differences in acute thermal tolerance are often present within fish populations, but the aspects of environmental change can mask the effects of genetic differences and thus the extent to which these traits undergo selection (Killen *et al.*, 2016). When considering correlations among repeatability, heritability, genetics and adaptive potential, it is critical to consider the influence of external factors on the physiology underlying  $CT_{max}$ . Individual phenotypic plasticity is context-dependent and changes over time, potentially hindering repeatability in natural settings (Claireaux *et al.*, 2013; Dingemans *et al.*, 2010; Killen *et al.*, 2016; Nussey *et al.*, 2007). The authors suggest that future studies focus on measuring the repeatability of  $CT_{max}$  under a range of environmental conditions.

### 3 | CONCLUSIONS

Assessing thermal tolerance has become a priority in the field of ecology, to predict potential impacts of global climate change (Figure 1). Understanding upper thermal limits is relevant not only to climate change but also to infrastructure planning, such as electricity generation, industry and stormwater management (*e.g.*, Turko *et al.* 2020; see Box 3 for research needs). This review advances the debate surrounding the ecological relevance of  $CT_{max}$ . Although  $CT_{max}$  has been criticized as an overly simplistic way of measuring

thermal tolerance, it remains an integrative metric with repeatable and comparable endpoints across individuals, populations and taxa. Moving forward, the use of a standardized protocol will be necessary to harmonize data and further advance the field (see Box 4 for a series of methodological considerations). In particular, a standardized protocol can be used to detect patterns within and across species, a task currently made difficult by the variability in protocols. With appropriate rates of heating, acclimation regimes across studies,  $CT_{max}$  can be repeatable and ecologically relevant, as well as comparable to other metrics of thermal tolerance. As researchers continue to evaluate how performance links to  $CT_{max}$ , they will be better able to determine the predictive power of CTM in forecasting responses to slow warming. Nonetheless,  $CT_{max}$  should not be considered a “silver bullet”; the understanding of the physiological mechanisms that lead to  $CT_{max}$ , LOE in particular, remains incomplete. Instead, it is argued that  $CT_{max}$  is a tool that should be used in combination with other indices to produce a more holistic description of thermal tolerance and thermal performance in fish. Field-based studies that integrate multiple approaches to measure thermal tolerance and performance in wild fish will likely yield the greatest insight. Our incomplete understanding of the physiological mechanisms that underlie thermal stress has resulted in “thermal tolerance” being only loosely defined. Temperature varies across time and space and, as such, predictions are reliant on complex multidimensional variation models. Perhaps unconsciously, researchers have used the broad definition of thermal tolerance with widely different approaches that often are not directly comparable to one another. There are now many opinions on what might constitute the “best” index of thermal tolerance. The most relevant approach will likely require careful contextualization to ensure that study objectives match the physiological performance responses selected, and this, in turn, will involve synthesizing mechanistic explanations because thermal stress acts on multiple levels of biological organization and differs across time scales.

### ACKNOWLEDGEMENTS

Funding for this project was made possible through an NSERC Discovery Grant awarded to Steven J. Cooke, Kathleen M. Gilmour and Amanda E. Bates. In addition, Kim Birnie-Gauvin was supported by the Villum Foundation. We would like to thank Keri E. Martin for providing feedback and edits that greatly improved the quality of the manuscript.

### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no data sets were generated or analysed during this study.

### ORCID

Jessica E. Desforges  <https://orcid.org/0000-0001-8089-4013>



## REFERENCES

- Allan, R. P., Hawkins, E., Bellouin, N., & Collins, B. (2021). *IPCC, 2021: summary for Policymakers*.
- Alfonso, S., Gesto, M., & Sadoul, B. (2021). Temperature increase and its effects on fish stress physiology in the context of global warming. *Journal of Fish Biology*, 98(6), 1496–1508.
- Andreassen, A. H., Hall, P., Khatibzadeh, P., Jutfelt, F., & Kersten, F. (2022). Brain dysfunction during warming is linked to oxygen limitation in larval zebrafish. *Proceedings of the National Academy of Sciences*, 119(39), e2207052119.
- Auer, S. K., Agreda, E., Chen, A. H., Irshad, M., & Solowey, J. (2021). Late-stage pregnancy reduces upper thermal tolerance in a live-bearing fish. *Journal of Thermal Biology*, 99, 103022.
- Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A., & Schulte, P. M. (2013). Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. *Journal of experimental biology*, 216(7), 1183–1190.
- Åsheim, E. R., Andreassen, A. H., Morgan, R., & Jutfelt, F. (2020). Rapid-warming tolerance correlates with tolerance to slow warming but not growth at non-optimal temperatures in zebrafish. *Journal of Experimental Biology*, 223(23), jeb229195.
- Bartlett, C. B., Garber, A. F., Gonen, S., & Benfey, T. J. (2022). Acute critical thermal maximum does not predict chronic incremental thermal maximum in Atlantic salmon (*Salmo salar*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 266, 111143.
- Bates, A. E., & Morley, S. A. (2020). Interpreting empirical estimates of experimentally derived physiological and biological thermal limits in ectotherms. *Canadian Journal of Zoology*, 98(4), 237–244.
- Baer, C. F., & Travis, J. (2000). Direct and correlated responses to artificial selection on acute thermal stress tolerance in a livebearing fish. *Evolution*, 54(1), 238–244.
- Becker, C. D., & Genoway, R. G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes*, 4(3), 245–256.
- Beitinger, T. L., & Bennett, W. A. (2000). Quantification of the role of acclimation temperature in temperature tolerance of fishes. *Environmental Biology of Fishes*, 58(3), 277–288.
- Beitinger, T. L., Bennett, W. A., & McCauley, R. W. (2000). Temperature tolerances of north American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes*, 58(3), 237–275.
- Beitinger, T. L., & Lutterschmidt, W. (2011). Measures of thermal tolerance. In: Farrell, A. (Ed.), *Encyclopedia of Fish Physiology: From Genome to Environment* (pp. 1695–1702). Academic Press.
- Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: A meta-analysis. *Animal Behaviour*, 77(4), 771–783.
- Blasco, F. R., Esbaugh, A. J., Killen, S. S., Rantin, F. T., Taylor, E. W., & McKenzie, D. J. (2020). Using aerobic exercise to evaluate sub-lethal tolerance of acute warming in fishes. *Journal of Experimental Biology*, 223(9), jeb218602.
- Boake, C. R. (1989). Repeatability: its role in evolutionary studies of mating behavior. *Evolutionary Ecology*, 3, 173–182.
- Bogan, M. T., Leidy, R. A., Neuhaus, L., Hernandez, C. J., & Carlson, S. M. (2019). Biodiversity value of remnant pools in an intermittent stream during the great California drought. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29(6), 976–989.
- Bouyoucos, I. A., Morrison, P. R., Weideli, O. C., Jacquesson, E., Planes, S., Simpfendorfer, C. A., ... Rummer, J. L. (2020). Thermal tolerance and hypoxia tolerance are associated in blacktip reef shark (*Carcharhinus melanopterus*) neonates. *Journal of Experimental Biology*, 223(14), jeb221937.
- Brandts, J. F. (1964). The thermodynamics of protein denaturation. I. the denaturation of chymotrypsinogen. *Journal of the American Chemical Society*, 86(20), 4291–4301.
- Chevin, L.-M., & Lande, R. (2010). When do adaptive plasticity and genetic evolution prevent extinction of a density-regulated population? *Evolution: International Journal of Organic Evolution*, 64(4), 1143–1150.
- Chen, Z., Anttila, K., Wu, J., Whitney, C. K., Hinch, S. G., & Farrell, A. P. (2013). Optimum and maximum temperatures of sockeye salmon (*Oncorhynchus nerka*) populations hatched at different temperatures. *Canadian Journal of Zoology*, 91(5), 265–274.
- Chen, Z., Snow, M., Lawrence, C. S., Church, A. R., Narum, S. R., Devlin, R. H., & Farrell, A. P. (2015). Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss* Walbaum). *The Journal of experimental biology*, 218(5), 803–812.
- Chown, S. L., Jumbam, K. R., Sørensen, J. G., & Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Functional Ecology*, 23(1), 133–140.
- Chung, K. (2001). Critical thermal maxima and acclimation rate of the tropical guppy *Poecilia reticulata*. *Hydrobiologia*, 462(1), 253–257.
- Claireaux, G., Théron, M., Prineau, M., Dussauze, M., Merlin, F.-X., & Le Floch, S. (2013). Effects of oil exposure and dispersant use upon environmental adaptation performance and fitness in the European sea bass, *Dicentrarchus labrax*. *Aquatic Toxicology*, 130, 160–170.
- Comte, L., & Olden, J. D. (2017). Climatic vulnerability of the world's freshwater and marine fishes. *Nature Climate Change*, 7(10), 718–722.
- Cowles, R. B., & Bogert, C. M. (1944). A preliminary study of the thermal requirements of desert reptiles. *Iguana*, 83, 53.
- Cox, D. K. (1974). Effects of three heating rates on the critical thermal maximum of bluegill. *Thermal Ecology*, 44, 158–163.
- Dahlke, F., Butzin, M., Wohlrab, S., & Pörtner, H.-O. (2022). Reply to: Methodological inconsistencies define thermal bottlenecks in fish life cycle. *Evolutionary Ecology*, 36(2), 293–298.
- Dahlke, F. T., Wohlrab, S., Butzin, M., & Pörtner, H.-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science*, 369(6499), 65–70.
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, 105(18), 6668–6672.
- Desforges, J. E., Birnie-Gauvin, K., Aarestrup, K., & Cooke, S. (2021). Upper thermal tolerance indicated by CTmax fails to predict migration strategy and timing, growth, and predation vulnerability in juvenile Brown trout (*Salmo trutta*). *Physiological and Biochemical Zoology*, 94(4), 215–227.
- DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in ecology & evolution*, 13(2), 77–81.
- Dillon, M. E., Wang, G., & Huey, R. B. (2010). Global metabolic impacts of recent climate warming. *Nature*, 467(7316), 704–706.
- Dingemanse, N. J., Kazem, A. J., Réale, D., & Wright, J. (2010). Behavioural reaction norms: Animal personality meets individual plasticity. *Trends in Ecology & Evolution*, 25(2), 81–89.
- Dochtermann, N. A., Schwab, T., & Sih, A. (2015). The contribution of additive genetic variation to personality variation: Heritability of personality. *Proceedings of the Royal Society B: Biological Sciences*, 282(1798), 20142201.
- Dohm, M. (2002). Repeatability estimates do not always set an upper limit to heritability. *Functional Ecology*, 16, 273–280.
- Dowd, W. W., King, F. A., & Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *The Journal of Experimental Biology*, 218(12), 1956–1967.
- Doyle, C. M., Leberg, P. L., & Klerks, P. L. (2011). Heritability of heat tolerance in a small livebearing fish, *Heterandria formosa*. *Ecotoxicology*, 20, 535–542.
- Ekström, A., Axelsson, M., Gräns, A., Brijis, J., & Sandblom, E. (2018). Importance of the coronary circulation for cardiac and metabolic performance in rainbow trout (*Oncorhynchus mykiss*). *Biology Letters*, 14(7), 20180063.

- Ekström, A., Gräns, A., & Sandblom, E. (2019). Can't beat the heat? Importance of cardiac control and coronary perfusion for heat tolerance in rainbow trout. *Journal of Comparative Physiology B*, 189(6), 757–769.
- Ekström, A., Jutfelt, F., & Sandblom, E. (2014). Effects of autonomic blockade on acute thermal tolerance and cardioventilatory performance in rainbow trout, *Oncorhynchus mykiss*. *Journal of Thermal Biology*, 44, 47–54.
- Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., ... Farrell, A. P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science*, 332(6025), 109–112.
- Elliott, J., & Elliott, J. (1995). The effect of the rate of temperature increase on the critical thermal maximum for parr of Atlantic salmon and brown trout. *Journal of Fish Biology*, 47(5), 917–919.
- Ern, R., Johansen, J. L., Rummer, J. L., & Esbaugh, A. J. (2017). Effects of hypoxia and ocean acidification on the upper thermal niche boundaries of coral reef fishes. *Biology Letters*, 13(7), 20170135.
- Ern, R., Norin, T., Gamperl, A. K., & Esbaugh, A. J. (2016). Oxygen dependence of upper thermal limits in fishes. *Journal of Experimental Biology*, 219(21), 3376–3383.
- Evans, D. O. (1990). Metabolic thermal compensation by rainbow trout: Effects on standard metabolic rate and potential usable power. *Transactions of the American Fisheries Society*, 119(4), 585–600.
- Fangue, N. A., Osborne, E. J., Todgham, A. E., & Schulte, P. M. (2011). The onset temperature of the heat-shock response and whole-organism thermal tolerance are tightly correlated in both laboratory-acclimated and field-acclimatized tidepool sculpins (*Oligocottus maculosus*). *Physiological and Biochemical Zoology*, 84(4), 341–352.
- Fangue, N. A., & Bennett, W. A. (2003). Thermal tolerance responses of laboratory-acclimated and seasonally acclimatized Atlantic stingray. *Dasyatis sabina*. *Copeia*, 2003(2), 315–325.
- Falconer, D. S. (1981). *Introduction to Quantitative Genetics* (2nd ed.). NY, USA: Longman.
- Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics* (4th ed.). Harlow, Essex, UK: Longmans Green.
- Frölicher, T. L., Fischer, E. M., & Gruber, N. (2018). Marine heatwaves under global warming. *Nature*, 560(7718), 360–364.
- Finnegan, S., Heim, N. A., Peters, S. E., & Fischer, W. W. (2012). Climate change and the selective signature of the late Ordovician mass extinction. *Proceedings of the National Academy of Sciences*, 109(18), 6829–6834.
- Genin, A., Levy, L., Sharon, G., Raitso, D. E., & Diamant, A. (2020). Rapid onsets of warming events trigger mass mortality of coral reef fish. *Proceedings of the National Academy of Sciences*, 117(41), 25378–25385.
- Goulet, C. T., Thompson, M. B., & Chapple, D. G. (2017). Repeatability and correlation of physiological traits: Do ectotherms have a “thermal type”? *Ecology and Evolution*, 7(2), 710–719.
- Goulet, C. T., Thompson, M. B., Michelangeli, M., Wong, B. B., & Chapple, D. G. (2017). Thermal physiology: A new dimension of the pace-of-life syndrome. *Journal of Animal Ecology*, 86(5), 1269–1280.
- Grinder, R. M., Bassar, R. D., & Auer, S. K. (2020). Upper thermal limits are repeatable in Trinidadian guppies. *Journal of Thermal Biology*, 90, 102597.
- Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, 282(1808), 20150401.
- Gilmour, K. M., & Bard, B. (2022). Social buffering of the stress response: insights from fishes. *Biology Letters*, 18(10), 20220332.
- Holland, W. E., Smith, M. H., Gibbons, J. W., & Brown, D. H. (1974). Thermal tolerances of fish from a reservoir receiving heated effluent from a nuclear reactor. *Physiological Zoology*, 47(2), 110–118.
- Huey, R. B., & Berrigan, D. (2001). Temperature, demography, and ectotherm fitness. *The American Naturalist*, 158(2), 204–210.
- Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A., Jess, M., & Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1596), 1665–1679.
- Illing, B., Downie, A., Beghin, M., & Rummer, J. (2020). Critical thermal maxima of early life stages of three tropical fishes: Effects of rearing temperature and experimental heating rate. *Journal of Thermal Biology*, 90, 102582.
- Isaza, D. F. G., Cramp, R. L., Smullen, R., Glencross, B. D., & Franklin, C. E. (2019). Coping with climatic extremes: Dietary fat content decreased the thermal resilience of barramundi (*Lates calcarifer*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 230, 64–70.
- Johansen, J. L., Nadler, L. E., Habary, A., Bowden, A. J., & Rummer, J. (2021). Thermal acclimation of tropical coral reef fishes to global heat waves. *eLife*, 10, e59162.
- Johnson, C. R. (1976). Diel variation in the thermal tolerance of *Gambusia affinis affinis* (Pisces: Poeciliidae). *Comparative Biochemistry and Physiology Part A: Physiology*, 55(4), 337–340.
- Jutfelt, F., Norin, T., Åsheim, E. R., Rowsey, L. E., Andreassen, A. H., Morgan, R., ... Speers-Roesch, B. (2021). ‘Aerobic scope protection’ reduces ectotherm growth under warming. *Functional Ecology*, 35(7), 1397–1407.
- Jutfelt, F., Roche, D. G., Clark, T. D., Norin, T., Binning, S. A., Speers-Roesch, B., ... Sundin, J. (2019). Brain cooling marginally increases acute upper thermal tolerance in Atlantic cod. *Journal of Experimental Biology*, 222(19), jeb208249.
- Killen, S. S., Adriaenssens, B., Marras, S., Claireaux, G., & Cooke, S. (2016). Context dependency of trait repeatability and its relevance for management and conservation of fish populations. *Conservation Physiology*, 4(1), cow007.
- Kingsolver, J. G., & Woods, H. A. (2016). Beyond thermal performance curves: Modeling time-dependent effects of thermal stress on ectotherm growth rates. *The American Naturalist*, 187(3), 283–294.
- Kumar, N., Ambasankar, K., Krishnani, K., Kumar, P., Akhtar, M., Bhushan, S., & Minhas, P. (2016). Dietary pyridoxine potentiates thermal tolerance, heat shock protein and protect against cellular stress of milkfish (*Chanos chanos*) under endosulfan-induced stress. *Fish & Shellfish Immunology*, 55, 407–414.
- Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of evolutionary biology*, 22(7), 1435–1446.
- LeBlanc, S., Middleton, S., Gilmour, K. M., & Currie, S. (2011). Chronic social stress is thermal tolerance in the rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*, 214(10), 1721–1731.
- Lefevre, S., Wang, T., & McKenzie, D. J. (2021). The role of mechanistic physiology in investigating impacts of global warming on fishes. *Journal of Experimental Biology*, 224(Suppl\_1), jeb238840.
- Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: History and critique. *Canadian Journal of Zoology*, 75(10), 1561–1574.
- Madeira, D., Narciso, L., Diniz, M. S., & Vinagre, C. (2014). Synergy of environmental variables alters the thermal window and heat shock response: An experimental test with the crab *Pachygrapsus marmoratus*. *Marine Environmental Research*, 98, 21–28.
- McArley, T. J., Hickey, A. J., & Herbert, N. A. (2017). Chronic warm exposure impairs growth performance and reduces thermal safety margins in the common triplefin fish (*Forsterygion lapillum*). *Journal of Experimental Biology*, 220(19), 3527–3535.
- McKenzie, D. J., Zhang, Y., Eliason, E. J., Schulte, P. M., Claireaux, G., Blasco, F. R., ... Farrell, A. P. (2020). Intraspecific variation in tolerance of warming in fishes. *Journal of Fish Biology*, 98(6), 1536–1555.
- Michelangeli, M., Goulet, C. T., Kang, H. S., Wong, B. B., & Chapple, D. G. (2018). Integrating thermal physiology within a syndrome: Locomotion, personality and habitat selection in an ectotherm. *Functional Ecology*, 32(4), 970–981.

- Morgan, R., Finnøen, M. H., Jensen, H., Pélabon, C., & Jutfelt, F. (2020). Low potential for evolutionary rescue from climate change in a tropical fish. *Proceedings of the National Academy of Sciences*, 117(52), 33365–33372.
- Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CT max is repeatable and doesn't reduce growth in zebrafish. *Scientific Reports*, 8(1), 1–8.
- Morgan, R., Sundin, J., Finnøen, M. H., Dresler, G., Vendrell, M. M., Dey, A., ... Jutfelt, F. (2019). Are model organisms representative for climate change research? Testing thermal tolerance in wild and laboratory zebrafish populations. *Conservation Physiology*, 7(1), coz036.
- Morley, S., Peck, L., Sunday, J., Heiser, S., & Bates, A. (2019). Physiological acclimation and persistence of ectothermic species under extreme heat events. *Global Ecology and Biogeography*, 28(7), 1018–1037.
- Mora, C., & Maya, M. F. (2006). Effect of the rate of temperature increase of the dynamic method on the heat tolerance of fishes. *Journal of Thermal Biology*, 31(4), 337–341.
- Munoz, M. M., Stimola, M. A., Algar, A. C., Conover, A., Rodriguez, A. J., Landestoy, M. A., & Losos, J. B. (2014). Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proceedings of the Royal Society B: Biological Sciences*, 281(1778), 20132433.
- Nati, J., Svendsen, M., Marras, S., Killen, S., Steffensen, J., Mckenzie, D. J., & Domenici, P. (2021). Intraspecific variation in thermal tolerance differs between tropical and temperate fishes. *Scientific Reports*, 11(1), 1–8.
- Nussey, D., Wilson, A., & Brommer, J. (2007). The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology*, 20(3), 831.
- O'Donnell, M. J., Regish, A. M., McCormick, S. D., Letcher, B. H., et al. (2020). How repeatable is CTmax within individual brook trout over short-and long-time intervals? *Journal of Thermal Biology*, 89, 102559.
- Payne, N. L., Morley, S. A., Halsey, L. G., Smith, J. A., Stuart-Smith, R., Waldo, C., & Bates, A. E. (2021). Fish heating tolerance scales similarly across individual physiology and populations. *Communications Biology*, 4(1), 1–5.
- Peck, L. S. (2011). Organisms and responses to environmental change. *Marine Genomics*, 4(4), 237–243.
- Penn, J. L., Deutsch, C., Payne, J. L., & Sperling, E. A. (2018). Temperature-dependent hypoxia explains biogeography and severity of end-Permian marine mass extinction. *Science*, 362(6419), eaat1327.
- Perry, A. L., Low, P. J., Ellis, J. R., & Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science*, 308(5730), 1912–1915.
- Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., & Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*, 569(7754), 108–111.
- Pottier, P., Burke, S., Drobniak, S. M., & Nakagawa, S. (2022). Methodological inconsistencies define thermal bottlenecks in fish life cycle: a comment on Dahlke et al. 2020. *Evolutionary Ecology*, 36(2), 287–292.
- Potts, L. (2020). *Hot and already bothered: Exploring effects of warming waters on an imperiled freshwater fish, Pugnose shiner Notropis anogenus*. MSc thesis. Montreal: McGill University.
- Recsetar, M. S., Zeigler, M. P., Ward, D. L., Bonar, S. A., & Caldwell, C. A. (2012). Relationship between fish size and upper thermal tolerance. *Transactions of the American Fisheries Society*, 141(6), 1433–1438.
- Rezende, E. L., & Bozinovic, F. (2019). Thermal performance across levels of biological organization. *Philosophical Transactions of the Royal Society B*, 374(1778), 20180549.
- Rezende, E. L., Castañeda, L. E., & Santos, M. (2014). Tolerance landscapes in thermal ecology. *Functional Ecology*, 28(4), 799–809.
- Rodgers, E. M., Todgham, A. E., Connon, R. E., & Fanguie, N. A. (2019). Stressor interactions in freshwater habitats: Effects of cold water exposure and food limitation on early-life growth and upper thermal tolerance in white sturgeon, *Acipenser Transmontanus*. *Freshwater Biology*, 64(2), 348–358.
- Rummer, J. L., Couturier, C. S., Stecyk, J. A. W., Gardiner, N. M., Kinch, J. P., Nilsson, G. E., & Munday, P. L. (2014). Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology*, 20(4), 1055–1066.
- Ryu, T., Veilleux, H. D., Munday, P. L., Jung, I., Donelson, J. M., & Ravasi, T. (2020). An epigenetic signature for within-generational plasticity of a reef fish to ocean warming. *Frontiers in Marine Science*, 7, 284.
- Sandblom, E., Clark, T. D., Gräns, A., Ekström, A., Brijis, J., Sundström, L. F., ... Jutfelt, F. (2016). Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nature Communications*, 7(1), 1–8.
- Saravia, J., Paschke, K., Oyarzún-Salazar, R., Cheng, C. C., Navarro, J. M., & Vargas-Chacoff, L. (2021). Effects of warming rates on physiological and molecular components of response to CTMax heat stress in the Antarctic fish *Harpagifer antarcticus*. *Journal of Thermal Biology*, 99, 103021.
- Sardella, B. A., Sanmarti, E., & Kültz, D. (2008). The acute temperature tolerance of green sturgeon (*Acipenser medirostris*) and the effect of environmental salinity. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 309(8), 477–483.
- Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, 218(12), 1856–1866.
- Schulte, P. M., Healy, T. M., & Fanguie, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*, 51(5), 691–702.
- Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, 5(1), 61–66.
- Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., ... Helmut, B. S. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters*, 19(11), 1372–1385.
- Somero, G. (2010). The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology*, 213(6), 912–920.
- Steinhausen, M., Sandblom, E., Eliason, E., Verhille, C., & Farrell, A. (2008). The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *Journal of Experimental Biology*, 211(24), 3915–3926.
- Stevens, E. D., & Fry, F. (1974). Heat transfer and body temperatures in non-thermoregulatory teleosts. *Canadian Journal of Zoology*, 52(9), 1137–1143.
- Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change*, 2(9), 686–690.
- Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T., & Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences*, 111(15), 5610–5615.
- Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C., & Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proceedings of the Royal Society B: Biological Sciences*, 274(1628), 2935–2943.
- Turko, A. J., Nolan, C. B., Balshine, S., Scott, G. R., & Pitcher, T. E. (2020). Thermal tolerance depends on season, age and body condition in imperiled reidside dace *Clinostomus elongatus*. *Conservation Physiology*, 8(1), coaa062.
- United States Environmental Protection Agency. (2022). Northwest Water Quality Temperature Guidance for Salmon, Steelhead and Bull Trout. Accessed: <https://www.epa.gov/wa/northwest-water-quality-temperature-guidance-salmon-steelhead-and-bull-trout>.
- Vander Vorste, R., Obedzinski, M., Nossaman Pierce, S., Carlson, S. M., & Grantham, T. E. (2020). Refuges and ecological traps: Extreme drought threatens persistence of an endangered fish in intermittent streams. *Global Change Biology*, 26(7), 3834–3845.

- Vertessy, R., Barma, D., Baumgartner, L., Mitrovic, S., Sheldon, F., & Bond, N. (2019). Independent assessment of the 2018–19 fish deaths in the lower Darling: Interim report, with provisional findings and recommendations.
- Vinagre, C., Dias, M., Cereja, R., Abreu-Afonso, F., Flores, A. A., & Mendonça, V. (2019). Upper thermal limits and warming safety margins of coastal marine species—indicator baseline for future reference. *Ecological Indicators*, *102*, 644–649.
- Vinagre, C., Leal, I., Mendonça, V., & Flores, A. A. (2015). Effect of warming rate on the critical thermal maxima of crabs, shrimp and fish. *Journal of Thermal Biology*, *47*, 19–25.
- Wang, G., & Dillon, M. E. (2014). Recent geographic convergence in diurnal and annual temperature cycling flattens global thermal profiles. *Nature Climate Change*, *4*(11), 988–992.
- Wang, T., Lefevre, S., Iversen, N. K., Findorf, I., Buchanan, R., & McKenzie, D. J. (2014). Anaemia only causes a small reduction in the upper critical temperature of sea bass: is oxygen delivery the limiting factor for tolerance of acute warming in fishes? *J. Exp. Biol.*, *217*, 4275–4278.
- Wegner, K. M., Kalbe, M., Milinski, M., & Reusch, T. B. (2008). Mortality selection during the 2003 European heat wave in three-spined sticklebacks: effects of parasites and MHC genotype. *BMC Evolutionary Biology*, *8*, 1–12.
- Wheeler, C. R., Lang, B. J., Mandelman, J. W., & Rummer, J. L. (2022). The upper thermal limit of epaulette sharks (*Hemiscyllium ocellatum*) is conserved across three life history stages, sex and body size. *Conservation Physiology*, *10*(1), coac074.
- West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford: Oxford University Press.
- Zanuzzo, F. S., Bailey, J. A., Garber, A. F., & Gamperl, A. K. (2019). The acute and incremental thermal tolerance of Atlantic cod (*Gadus morhua*) families under normoxia and mild hypoxia. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *233*, 30–38.
- Zhang, Y., & Kieffer, J. D. (2014). Critical thermal maximum (CTmax) and hematology of shortnose sturgeons (*Acipenser brevirostrum*) acclimated to three temperatures. *Canadian Journal of Zoology*, *92*(3), 215–221.

**How to cite this article:** Desforges, J. E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K. M., Eliason, E. J., Dressler, T. L., McKenzie, D. J., Bates, A. E., Lawrence, M. J., Fanguie, N., & Cooke, S. J. (2023). The ecological relevance of critical thermal maxima methodology for fishes. *Journal of Fish Biology*, *102*(5), 1000–1016. <https://doi.org/10.1111/jfb.15368>