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The ecological relevance of critical thermal maxima methodology for fishes

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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

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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°C min^{-1} or 18°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; Beitinger

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 *et al*. 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°C h^{-1} to 3.8°C min^{-1} (equivalent to 1.0°C day^{-1} to 5472.0°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 CT_{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°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°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 CT_{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). CT_{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). CT_{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, nonlethal 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 CT_{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 CT_{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 CT_{max} in fish ecology and by providing key directions for future research.

2 | EVALUATING THE ECOLOGICAL RELEVANCE OF CT_{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). CT_{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 CT_{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 CT_{max} should be estimated using more realistic heating rates that have greater

TABLE 1 Considerations for making critical thermal maximum (CT_{max}) research more ecologically relevant

Points of interest	Issues with the current situation	Recommendations to make CT_{max} more ecologically relevant
Thermal ramping	• Lack of consistent thermal ramping across studies	• Disclosure and validation of rate of thermal ramping
	• Ramping rate varies during experiments	• Rate of thermal ramping must be acute and ramped at a consistent rate until loss of equilibrium (LOE) is observed
	• Thermal ramping rate sometimes not reported	• Highlight more cases of acute thermal ramping in natural environments and study these species
	• Chronic rates are valid in many contexts but should not use the term CT_{max} to describe endpoints	
Acclimation	• Fish are not always acclimated long enough	• If attempting to compare CT_{max} endpoints to other studies for predictive purposes, ensure fish are fully acclimated to high temperatures using measurable indices.
	• Details of acclimation are not always disclosed	• Acclimate fish to different temperatures to determine the degree of phenotypic plasticity
	• CT_{max} is not comparable due to choice of acclimation temperature	• Report the duration of acclimation
Measures of repeatability and heritability	• Laboratory-derived estimates do not represent those that would be derived in the wild	• Perform field CT_{max} assays on wild fish to determine whether CT_{max} is repeatable under natural conditions
	• No evidence for evolutionary rescue	• Develop a greater understanding of underlying mechanisms involved in LOE. Use experimental evolution and artificial selection to test adaptation potential in diverse species
Thermal performance	• Few links between CT_{max} and performance indices	• Continue exploring the possibility of having a "thermal type" and how these relate to performance traits
	• Some frameworks (aerobic scope and CT_{swim}) are more useful to measure functional performance rather than CT_{max}	• Identify correlations between CT_{max} and alternative indices of thermal tolerance and performance

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_{max} (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^{-1}) 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_{max} 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_{max} specifically refers to a response derived from an *acute* thermal stress challenge (Lutterschmidt & Hutchison, 1997). Using the term CT_{max} to describe thermal tolerance derived using slow/chronic rates of thermal ramping adds variation to CT_{max} values reported in the literature and hinders the detection of patterns and efforts to use CT_{max} 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

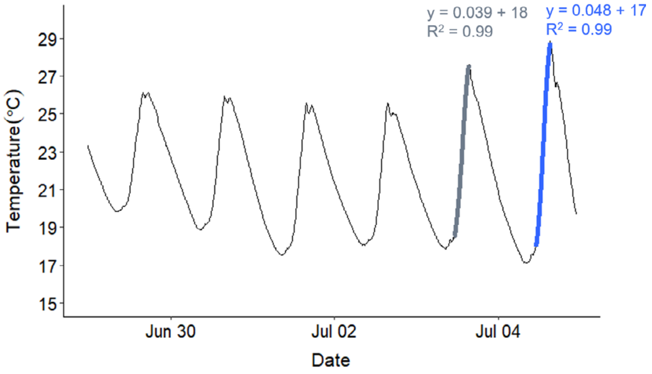
BOX 2 Case study on a population of *Oncorhynchus mykiss* living close to their upper thermal limit in Southern California

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^{-1}, respectively, well below the standard 0.3°C min^{-1} (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; Fangue 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 $\mathrm{CT}_{\max}$ 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 $\mathrm{CT}_{\max}$ or other methods.

Although acute upper thermal tolerance likely has a genetic component, stronger evidence of relationships between repeatability and heritability in $\mathrm{CT}_{\max}$ 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 (Dingemanse *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 $\mathrm{CT}_{\max}$ 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 $\mathrm{CT}_{\max}$ 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

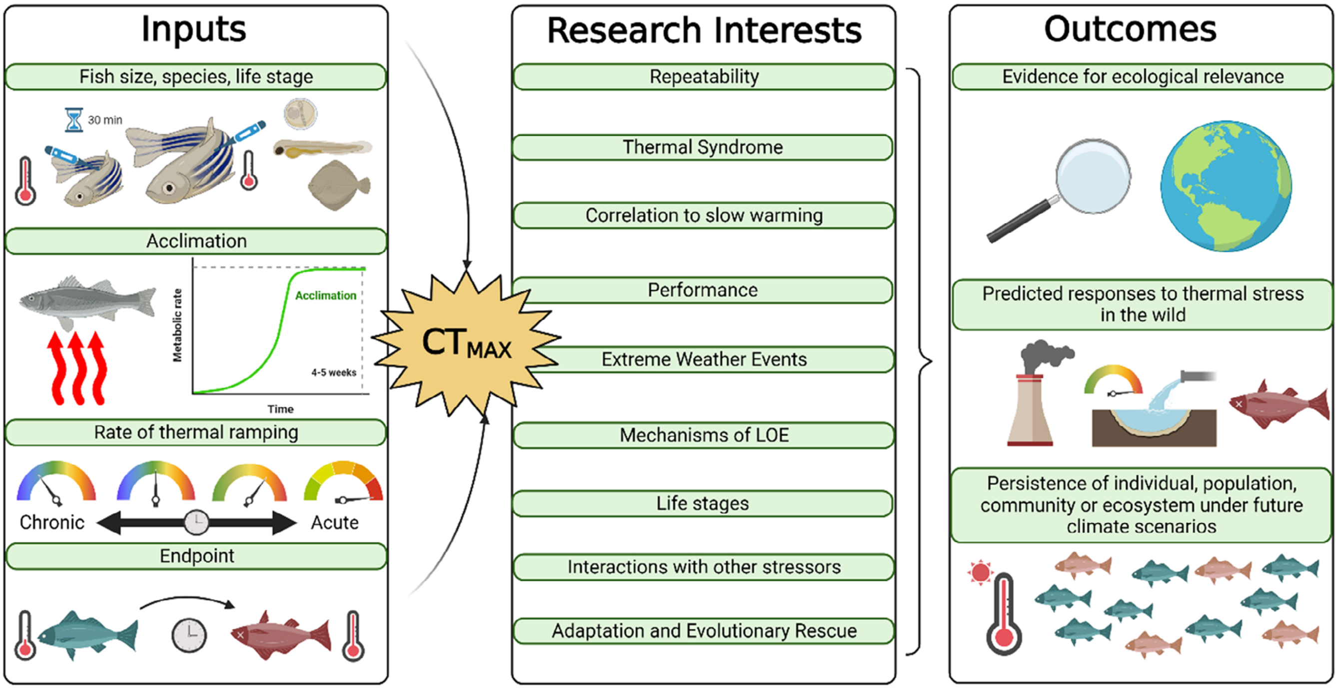


FIGURE 1 Conceptual diagram outlining the links between methodological inputs for estimating critical thermal maximum ($\mathrm{CT}_{\max}$), research interests and potential outcomes. $\mathrm{CT}_{\max}$ should be measured by considering important inputs, such as fish size, acclimation, rate of thermal 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_{max}**) **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_{max} 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_{max}. 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_{max}. 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_{maxima} 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_{max} 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_{maxima} 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_{max} 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 ± 0.008°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; Dingemanse *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.

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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.

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