The Ecological Relevance of Critical Thermal Maxima Methodology (CTM) for Fishes

Running title: Ecological Relevance of CTmax in 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. Here, we synthesize evidence to identify methodological concerns and common misconceptions that have limited the interpretation of CT_{max} (value for an individual fish during one trial) in ecological and evolutionary studies of fishes. We identify 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 various performance traits such as swimming ability, and repeatability. Care must be taken when interpreting CTM in ecological contexts, since 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 modeling species distribution, adaptation and/or performance in response to climate related temperature change. Our synthesis points to several key directions for future research that will further aid the application and interpretation of CTM data in ecological contexts.

Keywords: Upper thermal tolerance, ectotherms, thermal ecology, temperature, thermal stress, climate change

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I. Introduction : The History of CTM in fishes, past applications and limitations

Since its development in 1944 (Cowles and Bogert 1944), scientists have used critical thermal maximum method (CTM) as 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_{maxima} 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 and Bogert 1944). The simplicity of measuring CT_{maxima}, along with consistent behavioural responses at upper thermal limits across diverse taxa, have made CTM a popular choice in fisheries science since its inception (reviewed in Lutterschmidt and Hutchison 1997). Indeed, CTM assisted in the establishment of regulatory guidelines to manage thermal pollution from anthropogenic sources (e.g. Holland et al. 1974, EPA 2022: https://www.epa.gov/wa/northwest-water-quality-temperature-guidance-salmon-steelhead-and-bull-trout). As novel applications emerged, the original definition of CT_{max} evolved to include specifications regarding the importance of using consistent and acute heating rates, fish size, duration and temperature of the acclimation period, and the significance of thermal history (Lutterschmidt and 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 and Genoway 1979, Lutterschmidt and Hutchison 1997, Pottier et al. 2022).

The most widely accepted definition of CTmax 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 and Genoway 1979). LOE is one of the most prevalent responses to thermal stress (Lutterschmidt and Hutchison 1997) and is used as common (and non-lethal) end point for CTM testing. Following a recommended acute rate of thermal ramping to evaluate CT_{max} is critical for two main reasons: 1) it controls for discrepancies in temperatures between the water and the internal body of the fish, and 2) it prevents the modulation of physiological or biochemical pathways involved in inducing acclimation responses (Becker and Genoway 1979; Lutterschmidt and Hutchison 1997; Beitinger et al. 2000; Mora and Maya 2006). However, many studies used different CTM 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}) and in some studies the heating rates were not reported at all (Lutterschmidt and Hutchison 1997). These methodological differences limit ability to interpret and generalize results of CT_{max} in certain contexts.

Although a constant rate of temperature increase 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). However, using a uniform rate of 0.3°C min^{-1} for all contexts and species 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 better representations of thermal limits in fish. Similarly, methodological inconsistencies in measuring upper thermal tolerance across life stages has also led to much debate on the relevance CTM (Dahlke et al. 2020, Pottier et al. 2022, Dahlke 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. However, 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 derived from multiple studies.

Furthermore, standardizing a physiological endpoint (i.e., LOE) has similar limitations to those involved in using a prescribed 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, Wang et al. 2014; Lefevre et al. 2021). For instance, morphological or physiological differences in study organisms could alter the LOE response and lead to over- or under-estimated CT_{max} values. Fish of different age classes could 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 (Kumar et al. 2016; Zhang and Kieffer 2014; Gomez et al. 2019; McKenzie et al. 2020; O'Donnell et al. 2020). CT_{max} may also vary under different pH, salinity, and dissolved oxygen concentration regimes (e.g., Ern et al. 2016, Madeira et al. 2016, 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 and acute responses to thermal stress involving a sub-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. Here, we focus specifically on assessing the ecological relevance of using CT_{max} as a metric of thermal tolerance in fishes. In the following section, we present a series of questions regarding CTM and its ecological relevance. We also review important considerations for measuring and using CT_{max} in ecologically-relevant ways, and address how issues that arise during CTM can be avoided. We 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. We 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 key directions for future research.

II. Evaluating the ecological relevance of CT_{max} as a measure of upper thermal tolerance

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., Terblanche et al. 2007, Chown et al. 2009). Time is an important factor mediating responses to thermal challenges (i.e., 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 and 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, Ekström et al. 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., Wang et al. 2014, Ern et al. 2016, 2017, Jutfelt et al. 2019, Lefevre et al. 2021), different biological pathways may be involved in coping with acute versus chronic thermal stress (Peck 2011; Bates and Morley 2020, Lefevre et al. 2021). 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 and Morley 2020). Chronic thermal stress (days, weeks, 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 ecological relevance (e.g., Mora and Maya 2006, Vinagre et al. 2015, Bartlett et al. 2022). Very slow rates of warming (weeks to months) may be more representative of natural thermal challenges in some environments, and thus more likely to shape responses of fishes to warming climates (Vinagre et al. 2015, Bates and Morley 2020). 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} (Elliott and Elliott 1995; Beitinger et al. 2000). Others suggest that prolonged exposure to higher temperatures leads to cumulative thermal stress and lower thermal tolerance (Terblanche et al. 2007; Rezende et al. 2014).

A recent study by Åsheim et al. (2021) demonstrated a positive correlation between rapid (0.3°C min^{-1}) warming tolerance and slow warming (12 hour heating) tolerance in lab-reared zebrafish, indicating that similar processes could govern thermal tolerance. However, they subsequently examined growth rates at high temperatures, but failed to find a correlation whether they were challenged with a slow (over ~12h) or fast (0.3 °C min^{-1}) warming rate. This suggests that chronic responses to thermal stress are likely governed by different physiological processes than acute warming tolerance, since 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, in both Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*; Zanuzzo et al. 21019, Bartlett et al. 2022). Given these contrasting findings, it is difficult to determine whether slower rates 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 could provide valuable insight for how selection might occur in response to climate change, depending on the context or even the species (Åsheim et al. 2021, Bartlett et al. 2022).

Acclimation rates vary among species (Lutterschmidt and Hutchison 1997; Chung 2001; Vinagre et al. 2015), while 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 and Fry 1974). For these reasons, the effects of thermal ramping rates on CT_{max} and consequently, the ecological relevance of these rates, could vary among species. However, 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 and Hutchinson 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 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 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 and Morley 2020; Genin et al. 2020). While it fast rates of heating often overestimate functional thermal tolerance (Becker and 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 been caused by acute thermal shock in the wild (e.g., Wegner et al. 2008; Vertessy et al. 2019; Genin et al. 2020, Finnegan et al. 2012; Penn et al. 2018). 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 (IPCC 2021; Frölicher et al. 2018) could 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 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 be underpinned by fundamentally different mechanisms that limit thermal tolerance (such as protein denaturation versus oxygen or energy limitations, Brandts 1964; Jutfelt et al. 2021). Thus, despite the apparently higher ecological relevance of slow/chronic warming rates, the term CT_{max} should only be used when referring to upper thermal limits derived under acute rates of thermal ramping.

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 (Schulte et al. 2011; Johansen et al. 2021). 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 hemoglobin concentrations, spleen somatic index, and gill lamellar perimeter and width (Johansen et al. 2021). However, 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 et al. 1990).

Although it is widely accepted that acclimation influences upper thermal tolerance in fish (Beitinger and Bennett 2000; Schulte et al. 2011; Huey et al. 2012), the underlying physiological mechanisms remain poorly understood (Ern et al. 2016; McKenzie et al. 2020; Lefevre et al. 2021) 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 results across studies or perform meta-analyses or data syntheses with existing literature (Lutterschmidt and 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; Morgan et al. 2019; McKenzie et al. 2020). In wild zebrafish (*Danio reiro*) individuals living in warmer habitats had higher CT_{max}, likely due to acclimation (Morgan et al. 2019). It is therefore essential to critically assess acclimation temperatures and acclimation protocols across studies using CT_{max} when drawing conclusions about thermal limits. Additionally, the term 'acclimation' is frequently misused to refer to the relatively short adjustment period (also referred to as habituation) between when the organism is introduced into the CT_{max} apparatus and the start of the trial (Bates and 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 and Lutterschmidt 2011). While 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 and 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 cope better with climate change (Somero et al. 2009). In fact, adaptation can be accelerated by plasticity (West-Eberhard 2003; Lande 2009; Chevin and Lande 2010), which indicates 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 6 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 (Rummer et al. 2014; Wang and Dillon 2014; Morley et al. 2019; Ryu et al. 2020; Nati et al. 2021), although previous studies failed to find a link between plasticity and latitude or seasonality (Gunderson and 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. 2018). 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.

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 provide further insight into 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 could be explored. If CT_{max} could be linked to either increased or decreased performance, CT_{max} could 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 (TPCs) 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). Thermal performance curves 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 and Bozinovic 2019). Performance indices include behaviour, life-history traits, and physiological variables in ectothermic organisms (Rezende and 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 traveled per unit time, and enzyme reaction rates (Schulte et al. 2011). The increase in performance as temperatures reach optimal levels are 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 et al. 2011; Schulte 2015). The shape and breadth of TPCs can vary across levels of biological organization, as well as within and between species, according to seasonal patterns such as reproduction or migration, with phenotypic plasticity, geographic location, and time (Eliason et al. 2011; Schulte et al. 2011; Rezende and Bozinovic 2019). 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 (Schulte et al. 2011; Rezende and Bozinovic 2019).

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., Rezende et al. 2014, Dowd et al. 2015, Kingsolver and Woods 2016). For example, CT_{max} could 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 minutes. While 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 CTmax. 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}, they used a slow rate of ramping (1°C per 30 minutes) 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 could alter the LOE response and lead to over- or underestimated CT_{max} values. In lumpfish (*Cyclopterus lumpus)* for example, it could be particularly difficult to measure LOE because they have a suction disc that is used to attach strongly to rocks or other surfaces. For benthic fish, or those without a swim bladder, other endpoints include spiracle cessation (stingrays) onset of muscle spasms (Bouyoucos et al. 2020), or loss of righting response (experimenter disorients fish with probe and waits for re-righting, Fangue and Bennett 2003, Gervais et al. 2018, Andreassen et al. 2022). Understanding how LOE relates to alternative sub-lethal 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-dependant. 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., animal cannot avoid predation or seek cooler refuges). However, 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: Deutsche et al. 2008). Indeed, cumulative effects of sub-lethal and long-term temperatures may influence energy balance (Dillon et al. 2010), fecundity and developmental rates (Huey and Berrigan 2001), and ultimately fitness (Rezende and Bozinovic 2019). Upper thermal limits for physiological performance traits (e.g., aerobic scope, cardiac scope) differ from CT_{max}. However, CT_{max} remains useful as an index to compare against these upper thermal limits for performance traits, and can be applied at both the species and 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), since 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 are warm-tolerant (Goulet et al. 2017a). The notion of ‘types’ is based on a theoretical framework for studying correlated traits (at both inter- or intra-specific levels), and takes into consideration the links among temperature, metabolism and behaviour. Goulet et al. (2017a) 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 tolerance 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). However, more research would be required to determine whether alternative mechanisms of thermal tolerance could be organized into some form of thermal syndrome, as found in reptiles (Goulet et al. 2017a, b; Michelangeli et al. 2018; Åsheim et al. 2020).

CT_{max} was 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 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). Additionally, 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 (Rezende et al. 2014; Rezende and Bozinovic 2019; Lefevre et al. 2021). 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). However, CTM does not provide a way to assess impacts of longer exposures to sub-lethal temperatures on other organs (Lefevre et al. 2021). Organs could fail at similar temperatures, but across different durations of exposure (Lefevre et al. 2021). Some studies advocate for the use of an ecologically-relevant sub-lethal 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 sub-lethal threshold for tolerance of acute warming than LOE in fishes. However, 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). While TPCs are also useful methods to gain insight on thermal limits, they provide more information on how specific physiological systems react to thermal challenges.

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. , Deutsche 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. It is important to note that some studies use other metrics: 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 and Olden 2017). 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 and Olden 2017). Attempts have been made to account for this variability while modelling species distribution and predicting responses to climate change (e.g., Sunday et al. 2014; Comte and Olden 2017; Pinsky et al. 2019). 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 and Olden 2017).

Thermal safety margins can 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 (Wang and Dillon 2014; Frölicher et al. 2018) 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 compared to 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. However, 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 under-estimation of CT_{max} can lead authors to make incorrect inferences on thermal safety margins and species responses to warming when CT_{max} values are not adjusted according to 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.

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; Dohm 2002; Bell 2009). 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 and 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 (Dohm 2002; Bell 2009). 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 timescales, across life stages, 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 zero to one, where values closer to one 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 could have important benefits for range expansion or species redistribution, and improved coping with global climate change (Morgan et al. 2018). In addition to short timescales (days to weeks), others have found evidence of repeatability in fish over longer timescales. For example, O'Donnell et al. (2020) reported a repeatability coefficient of 0.48 for months to one year in brook trout (*Salvelinus fontinalis*) and

Grinder et al. (2020), 0.43 for six weeks in the Trinidadian guppy (*Poecilia reticulata*). Other studies that used alternative methods to measure upper thermal tolerance in fishes also have found evidence of heritability (e.g., Perry et al. 2005, Anttila et al. 2013, Muñoz et al. 2014, Muñoz et al. 2015), suggesting that thermal tolerance is (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 are still lacking. Because heritability is influenced by phenotypic variance, heritability could decrease under natural conditions owing to increased individual phenotypic plasticity (Nussey et al. 2007; Dingemanse et al. 2010; Killen et al. 2016). 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., Recsetar et al. 2012; Illing et al. 2020), diet (Isaza et al. 2019), water quality (e.g., Liddy and Wissing 1988, Sardella et al. 2008; Ern et al. 2016; Potts 2020), habitat (Rodgers et al. 2019), reproductive stage(Dalke et al. 2020, Johnson 1976, Auer et al. 2021, Wheeler et al. 2022), and social status (LeBlanc et al. 2011; Bard et al. 2020). Chronic stress in fish can also impair responses to thermal stress (e.g., LeBlanc et al. 2011; Claireaux et al. 2013; Bard et al. 2020). 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 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 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 (Falconer 1981; Dohm 2002; Dochtermann et al. 2015; Killen et al. 2016), and Morgan et al. (2018) found repeatability estimates to be greater than the heritability estimates from previous studies (e.g., Doyle et al. 2011; Meffe et al. 2011; Baer and Travis 2000). However, when there are significant genotype-environment interactions, repeatability may not always set upper boundaries for heritability (Dohm et al. 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 six 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 aspects of environmental change could 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 (Nussey et al. 2007; Dingemanse et al. 2010; Claireaux et al. 2013; Killen et al. 2016). We suggest that future studies focus on measuring the repeatability of CT_{max} under a range of environmental conditions.

Conclusions

Assessing thermal tolerance has become a priority in the field of ecology, in order to predict potential impacts of global climate change (Figure 1). Understanding upper thermal limits is not only relevant to climate change, but also to infrastructure planning, such as electricity generation, industry, and stormwater management (e.g. Turko et al. 2020). 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 5 for a series of methodological considerations). In particular, a standardized protocol could 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, comparable to other metrics of thermal tolerance. As we continue to evaluate how performance links to CT_{max}, we will be better able to determine the predictive power of CTM in forecasting responses to slow warming. However, CT_{max} should not be considered a 'silver bullet'; our understanding of the physiological mechanisms that lead to CT_{max}, LOE in particular, remains incomplete. Instead, we argue 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 and 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 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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Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

Tables

Table 1. Considerations for making 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 •Ramping rate varies during experiments • Thermal ramping rate sometimes not reported • Chronic rates are valid in many contexts but should not use the term CT_{max} to describe endpoints	• Disclosure and validation of rate of thermal ramping • Rate of thermal ramping must be acute and ramped at a consistent rate until LOE is observed •Highlight more cases of acute thermal ramping in natural environments and study these species
Acclimation	• Fish are not always acclimated long enough • Details of acclimation are not always disclosed • CT_{max} is not comparable due to choice of acclimation temperature	• If attempting to compare CT_{max} endpoints to other studies for predictive purposes, ensure fish are fully acclimated to high temperatures using measurable indices. • Acclimate fish to different temperatures to determine the degree of phenotypic plasticity • Report the duration of acclimation
Measures of repeatability and heritability	• Laboratory derived estimates do not represent those that would be derived in the wild • No evidence for evolutionary rescue	• Perform field CT_{max} assays on wild fish to determine whether CT_{max} is repeatable under natural conditions • 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 • Some frameworks (aerobic scope, CT_{swim}) are more useful to measure functional performance rather than CT_{max}	• Continue exploring the possibility of having a ‘thermal type’ and how these relate to performance traits • Identify correlations between CT_{max} and alternative indices of thermal tolerance and performance

Figures

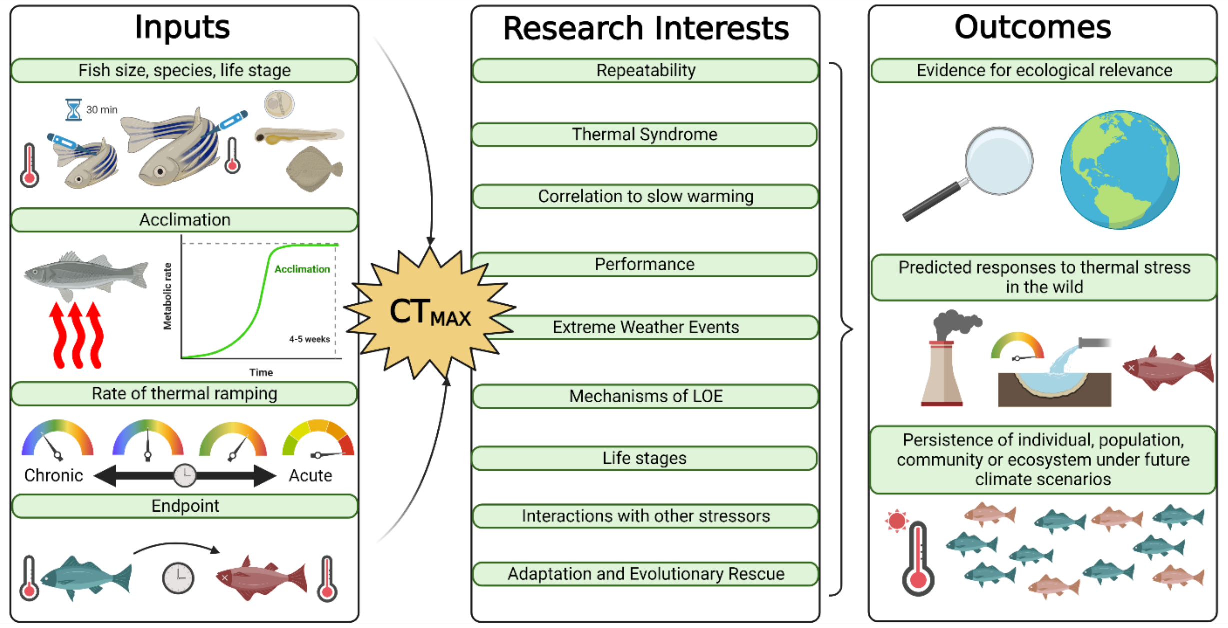

Figure 1. Conceptual diagram outlining the links between methodological inputs for estimating CT_{max}, research interests, and potential outcomes. CT_{max} should be measured by considering important inputs, such as fish size, acclimation, rate of thermal ramping, and an endpoint. Using an index of 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).

Boxes

Box 1. Glossary. *indicates the operational definition used for the purposes of this paper.

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 CTmax values obtained from a group of fish.
CTM	Critical thermal 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 and Morley 2020). *
Repeatability	Consistency of an individual’s performance over longer timescales, 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).

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, CT_{max} tests with rapid thermal ramping may be more ecologically relevant for species inhabiting these systems. In this case study, we deployed environmental data loggers in a stream that experienced extreme drying during the summer of 2021 in order to assess whether wild *Oncorhynchus mykiss* in southern California streams experience temperatures that approach their CT_{max} (~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, California, 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. In order 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 ~30 cm of water depth in July of 2021, temperatures reached 28°C and 29°C during the day (Fig. 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 two days before the logger dried out measured 0.039 and 0.048 °C/min respectively, well below the standard 0.3°C/min (Fig.1).

We 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 heatwaves 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.

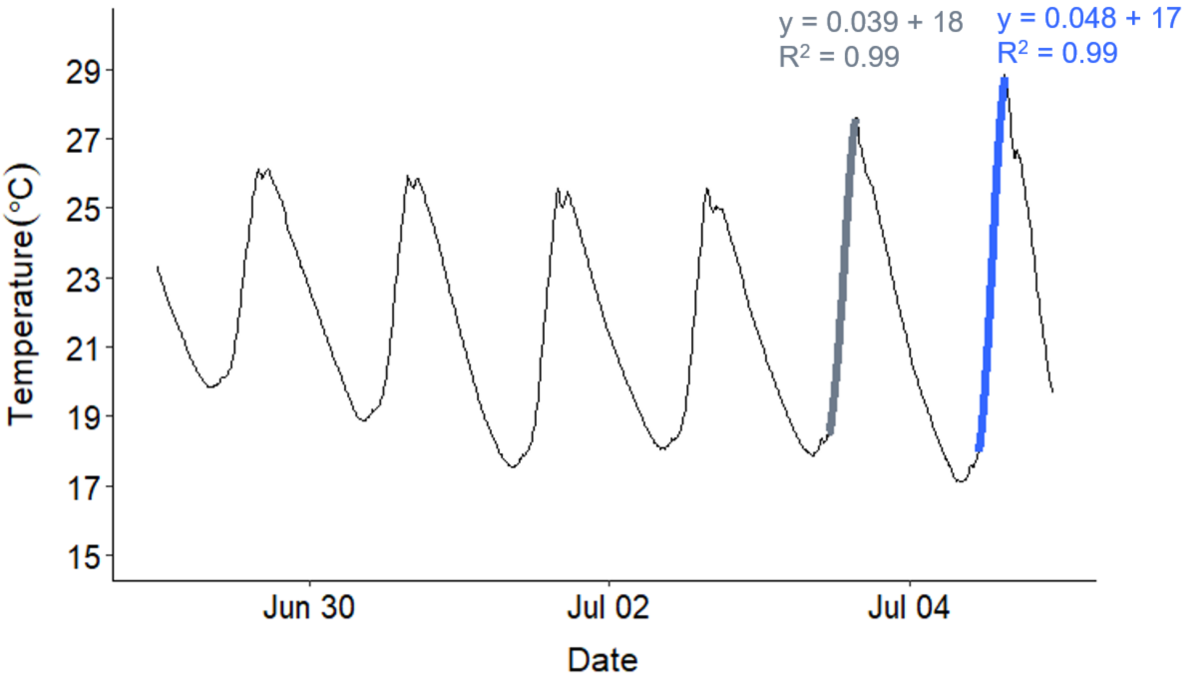

Figure 1. Temperature data from the submersible miniDOT logger (Precision Measurement Engineering, Vista, California, USA) for the final 5 days before the logger went dry (pool depth would have been ~30 cm when the logger was dry). Regression lines and corresponding equations represent the temperature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

Box 4. Research needs. We 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. We then generated a subsequent word cloud using these extracted words to narrow down the ten 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. We opted to add the term context, as it is central to research in the field of thermal biology. As such, we present the following list of nine themes to help focus research aimed at optimizing the use of CT_{max} in the context of ecology.

Research needs

Climate

Understanding how CTmax 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 CTmax 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 CTmax and survival, a proxy for Darwinian fitness.

Estimates

As with any metric of thermal tolerance, there is uncertainty associated with the underlying mechanisms of LOE, which is why they are considered estimates (with some uncertainty) of upper thermal limits. Reducing this uncertainty – either by standardizing protocols or integrating CTmax 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 CTmax to performance and fitness.

Conditions

Environmental conditions play an immense role in shaping thermal tolerance limits. Conducting CTmax 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 CTmax estimates, as they can be heavily influenced by rates of thermal ramping.

Acute, time, and rate

Future studies should acknowledge that CTmax 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 CTmax 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 CTmax.

Context

CTmax 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.

Box 5. Suggested series of considerations when performing CTM.

Considerations for CTM

1. **Define research goals.** CTM can be used in a variety of ways to answer a broad spectrum of research questions. While 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 critical aspects of the CTM such as fish size, origin, acclimation, and rate of ramping.
2. **Use the term "CTmax" with care.** Researchers should use this term with caution. While some variations in CTM are acceptable to meet the somewhat elusive standard, CTM should involve an acclimation period, an acute rate of ramping, and LOE or a widely accepted alternative sublethal endpoint. Chronic rates of ramping, lethal endpoints, and the use of performance indices rather than sublethal endpoints is 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**. While 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 acclimation.
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. While 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 CTmax. 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 subset 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. While 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.

References

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.

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.

Arias, P., Bellouin, N., Coppola, E., Jones, R., Krinner, G., Marotzke, J., Naik, V., Palmer, M., Plattner, G.-K., Rogelj, J., & others. (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group14 I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change; Technical Summary*.

Bard, B. (2020). Chronic Social Stress Impairs the Thermal Tolerance of Rainbow Trout *(Oncorhynchus mykiss)* [MSc Thesis]. Université d'Ottawa/University of Ottawa.

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.

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. L. (2011). Temperature | Measures of thermal tolerance. *Encyclopedia of Fish Physiology*, 1695-1702.

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.

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., Brauner, C. J., & 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.

Brett, J. R. (1971). Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerk*a). *American Zoologist*, *11*(1), 99–113.

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.

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 *Poecilla reticulata*. *Hydrobiologia*, *462*(1), 253–257.

Claireaux, G., & Lefrançois, C. (2007). Linking environmental variability and fish performance: Integration through the concept of scope for activity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*(1487), 2031–2041.

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.

Clark, T. D., Jeffries, K. M., Hinch, S. G., & Farrell, A. P. (2011). Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. *Journal of Experimental Biology*, *214*(18), 3074–3081.

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*, 158-163.

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.

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.

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.

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*, 273–280.

Donelson, J., Munday, P., McCormick, M., & Pitcher, C. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, *2*(1), 30–32.

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.

Ekström, A., Axelsson, M., Gräns, A., Brijs, 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., Brijs, J., Clark, T. D., Gräns, A., Jutfelt, F., & Sandblom, E. (2016). Cardiac oxygen limitation during an acute thermal challenge in the European perch: Effects of chronic environmental warming and experimental hyperoxia. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *311*(2), R440–R449.

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., Gale, M. K., Patterson, D. A., Hinch, S. G., & 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. and 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.

Farrell, A., Hinch, S., Cooke, S., Patterson, D., Crossin, G. T., Lapointe, M., & Mathes, M. (2008). Pacific salmon in hot water: Applying aerobic scope models and biotelemetry to predict the success of spawning migrations. *Physiological and Biochemical Zoology*, *81*(6), 697–708.

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.

Fry, F. (1971). The effect of environmental factors on the physiology of fish. *Fish Physiology*, 1–98.

Gervais, C. R., Nay, T. J., Renshaw, G., Johansen, J. L., Steffensen, J. F., & Rummer, J. L. (2018). Too hot to handle? Using movement to alleviate effects of elevated temperatures in a benthic elasmobranch, Hemiscyllium ocellatum. *Marine Biology*, *165*(11), 1–12.

Goulet, C. T., Thompson, M. B., & Chapple, D. G. (2017a). 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. (2017b). 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.

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.

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., Clark, T. D., & Speers-Roesch, B. (2021). 'Aerobic scope protection' reduces ectotherm growth under warming. *Functional Ecology*. *35*(7), 1397-1407.

Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., Lefevre, S., Nilsson, G. E., Metcalfe, N. B., Hickey, A. J., & others. (2018). Oxygen-and capacity-limited thermal tolerance: Blurring ecology and physiology. *Journal of Experimental Biology*, *221*(1), jeb169615.

Jutfelt, F., Roche, D. G., Clark, T. D., Norin, T., Binning, S. A., Speers-Roesch, B., Amcoff, M., Morgan, R., Andreassen, A. H., & 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).

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.

Larsson, S. (2005). Thermal preference of Arctic charr, *Salvelinus alpinus*, and brown trout, Salmo trutta–implications for their niche segregation. *Environmental Biology of Fishes*, *73*(1), 89–96.

LeBlanc, S., Middleton, S., Gilmour, K. M., & Currie, S. (2011). Chronic social stress impairs 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., Nati, J. J., & 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. (2020). Physiological plasticity and evolution of thermal performance in zebrafish. Doctoral Dissertation. NTNU.

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., Sarkar, K., & 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.

Munday, P. L., Crawley, N. E., & Nilsson, G. E. (2009). Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Marine Ecology Progress Series*, *388*, 235–242.

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.

Nilsson, G. E., Crawley, N., Lunde, I. G., & Munday, P. L. (2009). Elevated temperature reduces the respiratory scope of coral reef fishes. *Global Change Biology*, *15*(6), 1405–1412.

Nussey, D., Wilson, A., & Brommer, J. (2007). The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology*, *20*(3), 831–

Payne, N. L., Morley, S. A., Halsey, L. G., Smith, J. A., Stuart-Smith, R., Waldock, 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).

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.

Pörtner, H. O., & Farrell, A. P. (2008). Physiology and climate change. *Science*, 690–692.

Pörtner, H. O., & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, *315*(5808), 95–97.

Pörtner, H. O., & Peck, M. (2010). Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding. *Journal of Fish Biology*, *77*(8), 1745–1779.

Pörtner, H.-O. (2010). Oxygen-and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, *213*(6), 881–893.

Pörtner, H.-O., Bock, C., Knust, R., Lannig, G., Lucassen, M., Mark, F. C., & Sartoris, F. J. (2008). Cod and climate in a latitudinal cline: Physiological analyses of climate effects in marine fishes. *Climate Research*, *37*(2–3), 253–270.

Pörtner, H.-O., Bock, C., & Mark, F. C. (2017). Oxygen-and capacity-limited thermal tolerance: Bridging ecology and physiology. *Journal of Experimental Biology*, *220*(15), 2685–2696.

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. 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., & Fangue, 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.

Rodriguez-Lanetty, M., Wood-Charlson, E. M., Hollingsworth, L. L., Krupp, D. A., & Weis, V. M. (2006). Temporal and spatial infection dynamics indicate recognition events in the early hours of a dinoflagellate/coral symbiosis. *Marine Biology*, *149*(4), 713–719.

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., Brijs, J., Sundström, L. F., Odelström, A., Adill, A., Aho, T., & 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., Healy, T. M., & Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*, *51*(5), 691–702.

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.

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., Dong, Y., Harley, C. D., Marshall, D. J., Helmuth, B. S., & others. (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. Not in text

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.

United States Environmental Protection Agency. 2023. Northwest Water Quality Temperature Guidance for Salmon, Steelhead and Bull Trout. Accessed from : 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.

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.

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.