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Measuring maximum heart rate to study cardiac thermal performance and heat tolerance in fishes

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Summary statement: Maximum heart rate can be assessed in anaesthetized fish during acute warming to characterize cardiac thermal performance and upper thermal limits. The method is high throughput, and broadly applicable.

Abstract:

The thermal sensitivity of heart rate (f_{H}) in fishes has fascinated comparative physiologists for well over a century. We now know that elevating f_{H} is the primary mechanism through which fishes increase convective oxygen delivery during warming to meet the concomitant rise in tissue oxygen consumption. Thus, limits on f_{H} can constrain whole-animal aerobic metabolism. In this Review, we discuss an increasingly popular methodology to study these limits, the measurement of pharmacologically induced maximum f_{H} (f_{Hmax}) during acute warming of an anaesthetized fish. During acute warming f_{Hmax} increases exponentially over moderate temperatures (Q_{10} ~ 2-3), but this response is blunted with further warming (Q_{10} ~1-2) with f_{Hmax} ultimately reaching a peak ($Q_{10} \leq 1$) and the heartbeat becoming arrhythmic. Because the temperatures at which these transitions occur commonly align with whole-animal optimum and critical temperatures (e.g. aerobic scope and the critical thermal maximum) they can be valuable indicators of thermal performance. The method can be performed simultaneously on multiple individuals over a few hours and across a broad size range (<1g to >6000g) with compact equipment. This simplicity and high throughput make it tractable in lab and field settings and enable large experimental designs that would otherwise be impractical. As with all reductionist approaches, the method does have limitations. Namely, it requires anesthesia and pharmacological removal of extrinsic cardiac regulation. Nonetheless, the method has proven particularly effective in the study of patterns and limits of thermal plasticity and holds promise for helping to predict and mitigate outcomes of environmental change.

Introduction

The essential response of heart rate to thermal variation

The controlling influence of temperature on heart rate (f_H) has fascinated comparative physiologists for well over a century. Early interrogations of the relationship between temperature and biological rate functions often focused on f_H for practical and functional reasons (Crozier 1926; Cyon 1866; Fry 1947; Glaser 1929; Martin 1883; Zimmer 1998). From a practical perspective, the heartbeat could be directly observed at early life-stages in many species and in ex-vivo preparations, or readily assessed from recordings of blood pressure, blood flow, cardiac electrical activity and plethysmography. From a functional perspective, the vital role of f_H in meeting the oxygen requirements of aerobic metabolism and the sensitivity of f_H to temperature were well established (Fick 1870; Murlin and Greer 1914). These considerations remain highly relevant for fishes because we now know that elevating f_H is the primary mechanism through which fishes increase convective oxygen delivery to meet the inexorable rise in oxygen demand that occurs with acute warming. Beyond advancing basic knowledge, studying cardiac thermal performance has become increasingly important given the pressing need to understand species- and context-specific physiological responses to thermal variation in a rapidly changing world (Anttila et al. 2014a; Comte and Olden 2017; Eliason and Anttila 2017; Eliason et al. 2011; Eliason et al. 2013; Farrell 2016; Farrell et al. 2009).

When examining the mechanisms that shape an organism's ability to match its oxygen supply to the exponential rise in demand during warming, it is useful to consider each component of the Fick principal for oxygen uptake ($M□O_2$):

$$\dot{M}O_2 = f\mathrm{H} \cdot \mathrm{SV} \cdot (CaO_2 - CvO_2)$$

Where CaO_2-CvO_2 (oxygen content of arterial and venous blood, respectively) is the amount of oxygen extracted from circulating blood and the product of stroke volume (SV) and heart rate (f_H) is cardiac output (Q□). Trail-breaking research in fishes and other animals ranging from crustaceans to mammals has demonstrated that when hearts are warmed, resting and intrinsic f_H ($f_{H,rest}$ and $f_{H,intrinsic}$) initially increase with temperature coefficients similar to that for whole animal $M□O_2$ (Crozier 1926; Cyon 1866; Fry 1947; Glaser 1929; Henderson 1927; Knowlton and Starling 1912; Martin 1883). However, at high temperatures, f_H invariably reaches a plateau or decreases, with the heartbeat ultimately becoming arrhythmic causing a collapse in f_H. In fishes, SV does not appreciably increase with warming and can actually decline, thus limitations in f_H also constrain Q□ (Brodeur et al. 2001; Ekström et al. 2014; Eliason and Anttila 2017; Eliason et al. 2013; Farrell 2009; Gamperl et al. 2011; Steinhausen et al. 2008). Furthermore, fish can only partially offset limitations in Q□ by increasing the extraction of oxygen from the blood, because at low CvO_2 oxygen diffusion into vital tissues, including the myocardium, is limited (Ekström et al. 2016; Farrell and Clutterham 2003; Lannig et al. 2004). As such, the thermal limitations of f_H can impair whole animal M□O_2. A heat-induced cardiac collapse has now been documented in a broad range of fishes, including polar stenotherms like the Arctic cod (*Boreogadus saida*) (Drost et al. 2016b), notable eurytherms like the goldfish (*Carassius auratus*) and Atlantic killifish (*Fundulus heteroclitus*) (Ferreira et al. 2014; Safi et al. 2019) and numerous species in-between (Anttila et al. 2014a; Casselman et al. 2012; Chen et al. 2015b; Eliason and Anttila 2017; Eliason et al. 2011; Eliason et al. 2013).

Because f_{Hrest} increases with temperature, maintaining scope for f_H above f_{Hrest} to support vital functions including swimming and digestion (Eliason et al. 2013; Grans et al. 2009; Steinhausen et al. 2008) requires a proportional increase in f_{Hmax}. Fry (1947) first demonstrated that a fish can maintain or increase scope for f_H by increasing f_{Hmax} with acute warming, but only over temperatures that would be considered moderate for a given species. At warm temperatures f_{Hmax} increases to a lesser extent than f_{Hrest} and scope for f_H is lost before cardiac function collapses altogether (Fig. 1)(Eliason et al. 2013; Farrell 2009; Fry 1947; Steinhausen et al. 2008). Thus, the inability to increase f_{Hmax} with warming at high temperatures limits cardiac scope (scope for Q□), which constrains the aerobic metabolic scope (AS) available for functions beyond rest (Fry 1947). Based on this relationship, characterizing the thermal response of f_{Hmax} to acute warming can reveal temperatures at which sub-lethal and lethal limitations may restrict maximum tissue oxygen supply and AS. Such information is valuable for mechanistic predictions of how fish distributions will be affected by a rapidly changing world (Comte and Olden 2017; Pacifici et al. 2015).

These observations led Casselman *et al.* (2012) to develop a high-throughput method whereby pharmacologically induced f_{Hmax} is monitored in anaesthetized fish during acute warming to identify constraints on f_{Hmax} (referred to throughout as 'the method' or 'the f_{Hmax} method'). When the relationship between f_{Hmax} and temperature was expressed on an Arrhenius plot (natural log of the rate vs. the inverse of temperature in degrees Kelvin), they identified an initial breakpoint (Arrhenius breakpoint temperature; T_{AB}), above which the slope declined. This T_{AB} aligned well with the optimal temperature for the scope for f_H and aerobic scope, leading to the proposition that identifying T_{AB} could replace more laborious whole-animal assessments of AS in some circumstances. This f_{Hmax} method, with some modifications, is being increasingly used to study cardiac thermal performance – and heat tolerance more generally – in a broad range of scenarios. It has now been applied in >40 studies and across >20 species as a proxy for, or in compliment to, assessments of whole animal performances. In this Review, we provide an overview of this method and discuss its strengths, limitations, and application to assess cardiac thermal limits.

A method for rapidly screening maximum heart rate during acute warming

Overview of the method

As an overview, fish are placed under anaesthesia with assisted gill ventilation, fitted with electrocardiogram (ECG) electrodes and injected with drugs to block cardiac cholinergic tone and maximally stimulate adrenergic tone to a induce stable f_{Hmax}. Fish are then acutely warmed until the heartbeat loses rhythmicity (Fig. 2b-g). The analysis of the response of f_{Hmax} to acute warming yields multiple metrics that characterize cardiac thermal sensitivity and heat tolerance (Table 1; Fig.2e-g). The method was originally developed to test two fish simultaneously (Casselman et al. 2012). However, once proficient, users can increase to as many fish as can be practically managed. For instance, up to six fish have been assessed simultaneously (Adams et al. 2022; Gilbert et al. 2022b; Gilbert and Farrell 2021). While the protocol is conceptually simple, there are numerous considerations for new users, and for new species and contexts. These considerations are highlighted below.

Anaesthesia

Fish are immersed in a water bath at the fish's holding temperature containing an anaesthetic concentration sufficient to induce stage III anesthesia (i.e. cessation of bodily and opercular movement)(Coyle et al. 2004) in ~5 min. Anaesthetized fish are weighed during transfer to a sling immersed in a bath that recirculates a lower, maintenance concentration of anaesthetic over the gills which is continuously pumped via a mouthpiece inserted loosely into the mouth of the fish (using a cutoff large gauge needle, pipette tip or tubing, depending on fish size). The temperature of the maintenance bath can be lower than the holding temperature, particularly if multiple acclimation temperatures are being tested, so long as the temperature difference is not so large that it impacts subsequent response to warming (pilot tests should assess any concerns;(Gilbert and Farrell 2021; Safi et al. 2019). Tricaine methanesulfonate (also known as TMS, Tricaine, MS-222, and Ethyl 3-aminobenzoate) is the most commonly used anaesthetic and, as originally applied, does not appear to adversely impact the response of f_{Hmax} to warming (Casselman et al. 2012; see 'Critiques and limitations' below). Likewise, the assumption should be that appropriate anaesthetic concentrations vary among taxa and should be independently determined or verified in preliminary assessments. If the initial concentration is too high, ventilation can cease before the anaesthetic has equilibrated throughout important body compartments. The maintenance concentration of anaesthetic – typically between 50-80% the initial concentration – may also require pilot experiments. The guiding principle is to use the minimum concentration to prevent the resumption of opercular and body movement to ensure fish welfare, while not having excess anaesthetic that could impair heart function or be lethal. Some anaesthetics including TMS may require buffering of water pH.

Electrocardiogram

ECG electrodes are placed on anaesthetized fish in the holding sling. Electrode materials, placement and method of placement can all vary based on the specific experimental requirements and many options exist for the equipment and software to acquire ECGs. Pilot assessments are typically needed to determine the optimal electrode placement because it can vary substantially among species, life stages and recording modes. A precise electrode placement with less exposed recording surface is often required for small fish or in saltwater. Thus, for small fish, a needle electrode (e.g. MLA1213, ADInstruments, Colorado Springs, USA) affixed to a rod with only a small portion of the electrode exposed, can be gently placed on the ventral surface of a supine fish directly over the heart with a micromanipulator (Marchant and Farrell 2019; Safi et al. 2019). The second reference electrode can be placed more posteriorly on the body of the fish. For larger fish, needle electrodes or inexpensive small-gauge silver plated wire (e.g. 30AWG silver plated copper wire; R-30W-0050, Jonard Industries, Tuckahoe, NY) can be used and gently inserted in the skin on the ventral surface. In adults salmonids, for instance, electrodes can be placed diagonally across the heart on the ventral surface, with the reference electrode placed on the body or nearby in the bath as depicted by Cotter and Rodnick (2007). Recordings can be made as differential or single-ended as needed. High ECG quality and detail is needed to perform subsequent ECG waveform analyses and establish the exact type of arrhythmias observed at high temperatures.

Resources on common ECG acquisition, processing and analysis practices are available directly from equipment and software providers. Multiple amplifiers (e.g. Animal BioAmp, ADInstruments; DP-300 series, Warner Instruments, Warner Instruments, Hamden, CT, USA) and data acquisition platforms (e.g. PowerLab with Labchart software, ADInstruments; MP160 with AcqKnowledge software, BIOPAC Systems, Inc., Santa Barbara, CA, USA) are suitable for this application. Modern ECG acquisition configurations will generally have options for analog and digital filters. Analog filters are applied at the level of the amplifier and permanently modify the input signal, whereas digital filters are applied within the acquisition software and can be adjusted in real-time or after the signal is acquired. Most ECG information is acquired at between 1 and 50 Hz. Analog filters can be applied conservatively around this range (~0.1 to 100 Hz) to improve the signal-to-noise ratio while preserving all useful information. A digital band-pass filter then obtains a narrower range (~5 to 50 Hz) to reversibly improve signal quality so that ECG waveforms can be more clearly assessed. Some amplifiers and software have an optional 50 or 60 Hz Notch filter (or ‘mains filter’) that removes noise commonly associated with power line interference (line frequency varies by country). Finally, excessive electrical noise may be introduced by certain equipment in the bath or surrounding electronics. Suspected issues can be identified by briefly turning off electronics one at a time and once identified the equipment can be replaced or repositioned as needed.

Pharmacological interventions

Once water temperature and ECG recordings have stabilized (usually within a few minutes), f_{Hmax} is induced pharmacologically using intraperitoneal injections of the muscarinic-acetylcholine receptor antagonist atropine sulphate (to block parasympathetic inhibition of f_{H}), and a β-adrenoreceptor agonist such as isoproterenol (to mimic sympathetic acceleration of f_{H} via β-adrenoceptors). Alternative injection methods (e.g. intramuscular or intravascular) may be favoured in some scenarios such as with very small or very large fish, but the dosage, effect strength and effect duration should be verified before they are used. Under anesthesia and without these pharmacological interventions, the measured f_H cannot be considered as the resting, routine nor maximum level. Cholinergic (via the vagus nerve) and adrenergic tone (via circulating catecholamines and sympathetic innervation, if present) vary considerably among fishes and with acclimation temperatures (Wood et al. 1979; Axelsson et al. 1987; Altimiras et al. 1997; Axelsson 2005). Drug doses can vary with species, duration of the measurement period and temperature range. If appropriate dosages are not already established for a given scenario, pilot tests must be performed to determine these. Doses in the range of 1.2-10.0 mg kg^{-1} for atropine and 4.0-8.0 µg kg^{-1} for isoproterenol have been used successfully across a broad range of contexts (see *Data availability*). In some instances, isoproterenol has been omitted because it did not change f_{Hmax} after atropine injection (Anttila et al. 2014). Successful drug effects are indicated when a second, similar dose elicits no further change in f_H. Likewise, additional drug doses can be administered at any time during pilot tests, particularly near the end, to test whether the pharmacological effect has been maintained (Casselman et al. 2012). Alternative cardioactive drugs, applied following the same principles, can address other mechanistic questions. For instance, Marchant and Farrell (2019) used specific channel blockers to examine pacemaker mechanisms for f_{Hmax} under warming.

Warming rates

The original method used a warming rate 10°C h^{-1} for ~20 g fish (Casselman et al. 2012), applied in a stepwise manner (1°C step every 6 min). The first guiding principle was that the thermal increments ensured sufficient resolution (e.g. >10 data points) for precise identification of thermal limits without unduly prolonging test duration. For fish with limited warming tolerance above the experimental starting temperature, analytical resolution can be improved by using finer temperature increments (e.g. 0.5°C;(Drost et al. 2016b) over the same duration. The second guiding principle was to allow the heart temperature to equilibrate with the bath (Casselman et al. 2012), as indicated by f_{Hmax} stabilizing before the end of each 1°C increment. For ~20 g fish, Casselman et al. (2012) verified that a slower warming rate produced the same results. For small volumes of water, a typical lab heater-chiller device (Fig. 2) can achieve this heating rate. With larger fish, the water volume is larger, manually controlled heaters are added and slower warming rates are needed to increase temperature equilibration times (e.g. 5-6°C h^{-1}

in ~0.5 to >3 kg salmonids)(Gilbert et al. 2022b; Gilbert et al. 2020). Because gills are highly effective heat exchangers (Stevens and Sutterlin 1976) the blood supply returning to the heart may warm it faster than more insulated or less perfused body compartments like the peritoneal cavity where body temperature is commonly measured (e.g. Sandrelli and Gamperl 2023). The coronary circulation present in some fish also returns warmed blood directly from the gills to a portion of the heart. Thus, a stable f_{Hmax} may be a better indicator of cardiac temperature equilibration than measures of core body temperature – especially in large fish with coronary circulation. Nonetheless, future research on the topic could help refine the method and improve our general understanding of how fish experience acute thermal variation.

Data extraction and analysis

Real-time monitoring of f_{Hmax} is achieved through automated heartbeat or cyclic measurement detection algorithms in data acquisition software. The f_{Hmax} at each temperature increment is reported as an average over a specific period (e.g. 1 min, 30 s or 10 beats) towards the end of the increment. Thermal sensitivity (or dependence) of f_{Hmax} can be characterized in two ways. First, an Arrhenius breakpoint temperature (T_{AB}) can be identified for each individual fish using segmented regression analysis of the natural logarithm of f_{Hmax} against the inverse of temperature in kelvin (1/K)(Casselman et al. 2012)(Fig. 2F; Table 1). This regression is more accurate if the analysis is limited to temperatures over which f_{Hmax} is increasing. T_{AB} calculations are sensitive to the number of datapoints available and their distribution around the breakpoint. Insufficient data can be an issue if the fish's experimental starting temperature is close to or above T_{AB}. In this case, to increase the data available below T_{AB}, the starting temperature can be lowered (if confirmed that performance is not impaired), or a finer temperature increment can be used (e.g. 0.5°C).

A complementary or alternative analysis of the change in thermal sensitivity involves calculating the 'incremental Q_{10}', the Q_{10} temperature coefficient for every 1- 2°C of warming (Fig. 2G; Table 1). This incremental Q_{10} decreases with warming as f_{Hmax} approaches its peak and the temperature at which it falls below a specified Q_{10} threshold (T_{Q10}; (Anttila et al. 2013a) for the remainder of the trial can be used to summarize this decline. A Q_{10} threshold of ~1.9-2.0 is commonly selected to indicate a decreased thermal sensitivity since physiological rate functions during acute temperature changes typically have Q_{10} values ≥2. The T_{Q10} tends to be slightly (<1°C) higher than T_{AB} (Fig. 4C), but agreement depends on the selected Q_{10} threshold.

With warming above T_{AB} and T_{Q10}, f_{Hmax} reaches a plateau or a peak (Q_{10} ≤1), termed Peak f_{Hmax}, and the temperature is T_{PEAK} (Table 1). Further warming induces cardiac arrhythmia, at an upper thermal limit termed T_{ARR} (Fig. 2B-E; Table 1). Arrhythmia is generally unambiguous (Fig. S1), and identified as sudden intermittent drops in beat-to-beat f_{Hmax} (an irregular pattern of 3 or 2 heartbeats near the previous rhythmic frequency followed by a gap is common)(Anttila et al. 2013a; Casselman et al. 2012). Atrioventricular-block type arrhythmias are common and identified by the presence of a p-wave with a missing QRS complex if the ECG waveforms are analyzed (Fig. S1)(Gilbert et al. 2022a; Haverinen and Vornanen 2020; Vornanen 2020).

Thermal limits and performance metrics: interpretation and associations

The four thermal limits commonly identified using the f_{Hmax} method, in the order that they occur during warming, are T_{AB} or $T_{Q10,}$ $T_{PEAK,}$ and T_{ARR} (Table 1, Figs. 2, 4 and 5). These thermal limits represent transitions at which f_{Hmax} ($T_{PEAK,}$ and T_{ARR}) or the response of f_{Hmax} to warming (T_{AB} and T_{Q10}) become limited and are thus also called 'transition temperatures'. These metrics have ecological relevance to cardiac thermal performance, sensitivity, and tolerance and their definitions and suggested interpretations are summarized in Table 1. Below T_{AB}, for example, f_{Hmax} increases proportionally or to a greater extent with warming than f_{Hrest} (Fig. 1A). Thus, scope for f_H can be maintained to around T_{AB} but declines above T_{AB} (Fig. 1B). Also, T_{AB} can correspond with the optimal or upper pejus temperature for aerobic scope (Anttila et al. 2013b; Casselman et al. 2012; Chen et al. 2015b), although this is not always the case (Ferreira et al. 2014; Kraskura et al. 2023). Why these relationships vary among species and contexts is a natural avenue for future research.

Scope for f_H declines beyond T_{AB} and is low or negligible but T_{PEAK}. Thus, despite occurring at peak $f_{H,}$ T_{PEAK} does *not* correspond to the optimal temperature for performance. Rather T_{PEAK} lies beyond the optimal window for cardiac and aerobic capacity performance and is a temperature when fish are far more likely to be experiencing limitation of their maximal oxygen supply (Table 1). For this reason, and because of its close association with T_{ARR} (Fig 4 and 5) some studies end recordings at T_{PEAK} (Chen and Narum 2021). The difference between the f_{Hmax} at the acclimation temperature or the initial test temperature and peak f_{Hmax} is termed Δf_{Hmax} and provides information on the ability to increase f_{Hmax} (Fig. S3), helping to maintain a scope for f_{H} during acute warming, (i.e. the total thermal safety margin for f_{Hmax}; Fig. S3). Use of the term Δf_{Hmax} is preferable because 'scope' is more commonly defined as the difference between f_{Hmax} and f_{Hrest}. The final limit above T_{PEAK} is T_{ARR} which indicates a thermal limit for imminent cardiac failure and is beyond functional thermal limits of a fish.

The thermal limits values for f_{Hmax} are all positively correlated with the critical thermal maxima (CT_{MAX} ; Fig 4A), the upper temperature at which fish lose equilibrium. Understanding such relationships is useful because CT_{MAX} is the most commonly used metric to characterize whole-animal heat tolerance (Desforges et al. 2023). When CT_{MAX} and f_{Hmax} values compiled from available studies (see '**Data availability**') were compared we found that T_{ARR} was 11% below CT_{MAX}, T_{PEAK} was 7% below T_{ARR}, and T_{Q10} and T_{AB} were ~25% lower than T_{PEAK} (Fig. 5). CT_{MAX} is, however, commonly assessed using higher warming rates (typically 0.3°C min^{-1}), which can affect such comparisons. Nonetheless, T_{ARR} is generally similar to or slightly below CT_{MAX}. The other thermal limit metrics for f_{Hmax} are typically well below CT_{MAX} (Fig 5B and 6D; see Data Availability) and at temperatures that are inherently encountered more often in the wild, thereby improving their direct ecological relevance. Conversely, temperatures as high as CT_{MAX} or T_{ARR} are acutely lethal which has required species to evolve behavioural avoidance strategies and occupy biogeographical distributions that make exposure to such temperatures rare (Payne et al. 2016). Below these critical temperatures, the T_{PEAK} and T_{Q10} and T_{AB} are generally sub-lethal temperatures, and indicate a form of f_{Hmax} limitation that that can impair or impose trade-offs on the performance of fitness related functions (e.g. swimming performance and feeding) (Table 1). While limits to f_{Hmax} such as T_{peak} may be important in many contexts, other vital processes (e.g. feeding or digestion rate) may independently become constrained at lower temperatures and over different time-scales, all of which should be considered when making inferences about the effects of temperature on whole-animal performance.

Strengths

A crucial requirement to make predictions for how a warmer future might affect the distribution and success of fish populations is reliably characterizing their upper thermal limits over varied timescales (Comte and Olden 2017; Desforges et al. 2023). Whole-animal CT_{MAX} has a long history and is technically simple to perform (Desforges et al. 2023). Hence CT_{MAX} data are widely available, facilitating impactful examinations of biogeographical patterns of thermal tolerance and important comparisons of upper thermal limits among and within species (e.g., Comte and Olden 2017; Sunday et al. 2011). Yet, the direct ecological relevance of CT_{MAX} continues to be debated, outside the context of relatively rare acute warming events that can cause mass mortality (Desforges et al. 2023). In contrast, characterizations of thermal performance curves for traits like growth rate and aerobic scope have strong ecological relevance and have been used to set water temperature criteria by fisheries managers (Eliason et al. 2024). However, these measurements are technically more challenging and far more time-consuming than determining CT_{MAX}, making them challenging to perform over broad ranges of species, life stages and environmental contexts. With the rapid rate of ongoing environmental change and over 32,000 species of fishes, the f_{Hmax} method provides an intermediate between more detailed, laborious approaches (Aerobic scope) and coarser high-throughput methods (CTmax) while still providing valuable information relevant to thermal tolerance and limits to cardiorespiratory performance. Ultimately, effective conservation and management practices (e.g. setting water quality targets) are rarely based on single studies or narrow lines of evidence. Rather, multiple lines of evidence are weighed and synthesized to frame conservation challenges and potential interventions (Mayer et al. 2023). To this end, we recommend the f_{Hmax} method as part of the toolbox.

The f_{Hmax} method is a high-throughput assessment of a several ecologically relevant thermal limits and specific information on cardiorespiratory thermal performance. Depending on the warming rate and temperature range, 12 fish can be comfortably assessed in a day with an entire protocol taking ~2-5 h for 2-6 fish. The method's other advantages include that it is simple to perform, highly mobile and amenable for field studies in remote locations (Drost et al. 2014; Gilbert et al. 2020; Hansen et al. 2016). Indeed, field-based ECG recordings and data presented here (Fig. 2b-g, Fig. S1) demonstrate the ability to obtain laboratory quality data in remote settings (Gilbert et al. 2022a). Drost et al. (2014) directly compared fish tested in a field and laboratory setting and obtained similar quality data and typical responses in both cases. However, thermal acclimation conditions differed between the field and lab precluding direct comparison of absolute values. The method's strengths enable studies with large or complex designs (see Applications section) or have tight time constraints (e.g. a brief field trip or window of fish availability). Successful applications also include a broad range of species, life-stages and body masses (e.g. ~0.5 g Zebrafish to >3 kg adult Arctic char) (Gilbert et al. 2022a; Marchant and Farrell 2019)(see '**Data availability**'). Note that a study of Arctic cod (*Boreogadus saida*), demonstrated the potential for the method to be applied with larvae but required significant methodological adjustments (Drost et al. 2016a) and so further validation of TMS effects and pharmacological interventions are needed before widespread with such early life stages.

The use of pharmacological interventions and anesthesia give rise to some benefits but also have some limitations (see 'Critiques and limitations'). Anaesthetized fish provide a minimally invasive alternative to more invasive methods that assess cardiorespiratory performance, a marked benefit for fish welfare. Also, fewer fish are needed to generate equivalent data using the f_{Hmax} method. In non-anaesthetized fish, 10 acute exposure temperature challenges with a typical sample size of 8 requires either 80 fish, or repeatedly exposing, exercising and recovering the same 8 fish each 10 times, which raises additional fish welfare concerns. In contrast, eight anaesthetized fish can generate similar data in a single day using the f_{Hmax} method (see 'Overview' section; e.g., Gilbert and Farrell 2021 and Hardison et al 2023) .

Furthermore, anesthesia eliminates behavioural responses to warming, limiting the associated variation in f_{H} and ECG quality. Likewise, variation in the autonomic regulation of $f_{\text{H},}$ (Casselman et al. 2012) is eliminated by artificial stimulation of f_{Hmax}. These pharmacological interventions also mean that fish can be assessed without a prolonged recovery after capture – an important consideration for field studies. The information gained from the f_{Hmax} method can subsequently help streamline studies of other aspects of cardiorespiratory function. Together these strengths make the f_{Hmax} method highly useful for both basic and applied research on the thermal limits to cardiorespiratory performance.

Critiques and Limitations

All reductionist approaches have limitations, some of which affect how useful the results are for understanding whole-animal function. Such methods, however, are most useful when users recognize these limitations and apply the methods for suitable purposes (Treberg et al. 2020). The f_{Hmax} method focuses specifically on f_{Hmax} because of the central role of f_{H} in supporting whole-animal aerobic capacity and the cardiovascular response to acute warming

(Casselman et al. 2012; Eliason et al. 2013). The f_{Hmax} method reveals the upper limits for f_{H} during acute warming. It does not, nor is it intended to, reflect the response of f_{Hrest} to acute warming, except perhaps at high temperatures where the two responses converge (Fig. 1). This explicit intent has been overlooked in some critique of the approach (Porter and Gamperl 2023; Sandrelli and Gamperl 2023). Alternate methods exist to measure f_{Hrest}, but measuring f_{Hrest} may not distinguish between a vagal slowing of f_{Hrest}, which may be a protective mechanism (Eliason et al. 2013) from cardiac impairment due to arrhythmia.

Anaesthesia impairing f_{Hmax} (and its response to warming) is a concern, as examined experimentally and discussed by Casselman *et al* (2012). Certainly, f_{Hrest} is commonly affected by anaesthesia (Cotter and Rodnick 2006), but such studies rarely distinguish between direct effects of anaesthetics on f_{H} or cardiac function (Haverinen et al. 2018) and indirect effects. Indirect effects arise from the partial blockade of vagal tone by some anaesthetics, a release of catecholamines (Lochowitz et al. 1974; Randall 1962) or hypoxemia driven by the decrease in ventilation which then triggers vagal slowing of f_{H}. These indirect effects are not a factor when using the f_{Hmax} method because vagal tone is blocked and the gills are artificially ventilated with well oxygenated water. All the same, the usual anesthetic used (TMS) is a sodium channel antagonist and can impair cardiac sodium currents at sufficient concentrations (Haverinen et al. 2018). In zebrafish cardiomyocytes, Haverinen et al. (2018), found a reduction in sodium current at TMS concentrations >168 mg L^{-1} but none below ~100 mg L^{-1}. This higher TMS concentration is above the maintenance concentration commonly used for the f_{Hmax} method and available evidence for salmonids suggests minimal if any effect of a maintenance concentration of TMS on f_{Hmax} (Casselman et al. 2012). Not surprisingly then, the response of f_{H} in non-anaesthetized atropinized rainbow trout to acute warming (Gilbert et al. 2019) was nearly identical to that for the f_{Hmax} method (Fig. 3). Nonetheless, researchers should use a minimal TMS maintenance concentration to avoid untoward effects (as above). Unfortunately, equivalent data are unavailable for other species.

A related concern is that anesthesia abates any stress responses and associated additional metabolic demands that normally arise at high temperature. The fish’s internal milieu may constrain f_{Hmax} perhaps through cardiac oxygen or substrate limitations that do not occur to the same extent *ex vivo*, in fish at rest or while under anesthesia (Eliason et al. 2013). Indeed, while f_{Hmax} was indistinguishable for non-anaesthetized and TMS-anaesthetized fish over intermediate temperatures, f_{Hmax} tended to be lower in non-anaesthetized fish forced to exercise near their upper thermal limits (Casselman et al. (2012). In non-anaesthetized sockeye salmon (*Oncorhynchus nerka)* at high temperatures, f_{Hmax} during exercise fell below the f_{Hrest} of non-exercising fish (Eliason et al. 2013). Also, peak f_{Hmax} is substantially reduced under hypoxia (Schwieterman et al. 2023). Thus, the f_{Hmax} method may produce a 'best case scenario' for the response to acute warming. Congruently, warming induced peak f_{H} in non-exercised, non-anaesthetized rainbow trout (*Oncorhynchus mykiss*) and Arctic char (*Salvelinus alpinus*) is highly similar to that from the f_{Hmax} method (Fig. 3). Nonetheless, T_{ARR} is generally less than – or occasionally similar – to CT_{MAX} (Fig. 3) with the other thermal limits falling below T_{ARR}, so the method still provides a series of conservative estimates of acute heat tolerance.

While eliciting f_{Hmax} pharmacologically has the concern that it precludes normal CNS integration to warming, the benefits of autonomic control of f_{H} to both cardiac and whole-animal thermal tolerance have been explored in non-anaesthetized fish (Ekstrom et al. 2021; Gilbert et al. 2019). Stimulation of cardiac *ß*-adrenergic receptors can improve heat tolerance and increase peak f_{H} (Ekstrom et al. 2021; Gilbert et al. 2019); the f_{Hmax} method achieves this by injection of isoproterenol rather than a CNS-induced increase in sympathetic output to the heart. Blocking muscarinic acetylcholine receptors with atropine, however, can prevent the increase T_{PEAK} (Gilbert et al. 2019), but does not always (Ekström et al. 2014; Ekstrom et al. 2021). Even if f_{Hmax} values from anaesthetized fish are generally consistent with available literature values (see (Anttila et al. 2013a; Casselman et al. 2012), further direct investigations comparing pharmacologically and activity induced f_{Hmax} in anesthetized and non-anaesthetized fish respectively, would help quantify effects of anesthesia. To this end, Sandrelli and Gamperl (2023) compared f_{H} responses to acute warming in anaesthetized fish, non-anaesthetized fish confined in a respirometer and free-swimming fish. They discovered multiple differences in f_{H} and cardiac heat tolerance among the different methods. They applied pharmacological treatments similar to those discussed here in anaesthetized fish, however f_{Hmax} was not measured in non-anaesthetized. Other differences precluded direct evaluation of the f_{Hmax} method including an invasive surgical implantation of ECG loggers, high initial anesthetic concentration, caudal vein injections of the cardioactive drugs, a warming rate that the authors determined was too fast for the large fish used in the study, and a continuous warming ramp or large warming increment, although the specific details are not presented. Furthermore, intermittent ECG recordings precluded the identification of $T_{ARR.}$ Nevertheless, future studies aiming to evaluate and refine the f_{Hmax} method or complementary high-throughput methods are welcome given the urgent need to expand our knowledge of species- and context-specific aspects of thermal physiology.

Applications of the measurement of f_{Hmax} in anaesthetized fish

Characterization of cardiac thermal performance and heat tolerance

The initial proposed application of the f_{Hmax} method was to rapidly assess T_{AB} as a proxy for the optimal temperature for AS because of their numerical associations (Anttila et al. 2013a; Casselman et al. 2012). It continues to be applied in that regard, but its use has quickly expanded to include assessments of upper thermal limits and the general study of cardiac thermal responses to acute warming. It has been used to study cardiac thermal performance in data-poor-species (Hansen et al. 2016) (Drost et al. 2014) (Skeeles et al. 2020), to determine how that performance relates to environmental exposures under current and climate change scenarios (Gilbert et al. 2020) (Van Der Walt et al. 2021) and to examine associations among physiological functions across levels of organization (Anttila et al. 2013a) Strowbridge et al. 2024)(Adams et al. 2022).

Examining intra- and interspecific diversity of thermal physiology

As the application the f_{Hmax} method expands to a broader range of species and contexts, opportunities emerge for broadscale examinations of phylogenetic and biogeographical patterns in cardiac thermal performance as previously explored for AS and CT_{MAX} (Comte and Olden 2017; Payne et al. 2016; Sunday et al. 2019; Sunday et al. 2011). While outside the specific scope of this Review, the compiled data (‘**Data availability**; Fig. S2) demonstrates that sufficient data are already available to allow for interspecific comparisons of thermal physiology among species or other levels of classification. However, such interspecific comparisons within a single study are currently uncommon. One study did identify differences in cardiac thermal tolerance among closely related *Danio* species (Sidhu et al. (2014). More studies have applied the method to examine intraspecific variation in thermal physiology among genetic crosses, and strains within multiple salmonid species (Anttila et al. 2014a; Chen et al. 2013; Chen et al. 2018a; Chen et al. 2018b; Chen and Narum 2021; Chen et al. 2015b; Gradil et al. 2016; Muñoz et al. 2014a; Muñoz et al. 2014b); Strowbridge et al. 2024*)*. For instance, (Chen et al. 2018b) found that even when reared in a common environment, redband trout (*Oncorhynchus mykiss*) from populations obtained from cool montane habitats had a lower peak f_{Hmax} than a population obtained from a hot desert environment. This difference corresponded with population differences in aerobic thermal performance and gene expression.

Examining context dependence of cardiac thermal performance and heat tolerance

The method has also been used to examine the effect of numerous other factors on cardiac thermal performance with both basic science and conservation motives. Kraskura et al. (2023) found that, as expected, f_{Hmax} decreased as body mass increased (mass scaling exponent: -0.05), whereas cardiac thermal limits increased with body mass. Others have found that the specific nutrients and dietary compounds (e.g. taurine and fucoidan) can affect peak f_{Hmax} and cardiac thermal performance (Baker et al. 2023; Dixon et al. 2023; Papadopoulou et al. 2022), as with diet more generally (Hardison et al. 2021; Hardison et al. 2023). Researchers have also examined associations with swimming performance (Anttila et al. 2014b) and identified improved cardiac heat tolerance following exercise training in some contexts (e.g., intermediate exercise intensity)(Papadopoulou et al. 2022; Pettinau et al. 2022b). Other factors examined have included contaminant exposures (Anttila et al. 2017), genetic modification (Chen et al. 2015a), induction of triploidy (Verhille et al. 2013), varied life-history tactics (Mottola et al. 2020), hypoxia and hyperkalemia (Schwieterman et al. 2023), ocean acidification (Crespel et al. 2019), and thermal history (Eliason and Anttila 2017). Among these, the method has been most widely and effectively applied in the study of patterns and limits in cardiac plasticity in response to varied thermal histories.

Cardiac thermal plasticity

Studies of thermal physiology often struggle to separate the consequences of temperature acclimation from the direct effect of thermal variation. A principal strength of the f_{Hmax} method is that this separation can be made straightforward by examining the cardiac effects of acute thermal change in fish from multiple acclimation temperatures. Thermal acclimation can reset the intrinsic cardiac pacemaker rate (through changes in membrane/ion channels and pumps) and change the level of autonomic control of heart rate (Gamperl and Farrell 2004; Sutcliffe et al.

2020; Vornanen et al. 2002a; Vornanen et al. 2002b). Such changes are revealed in the f_{Hmax} method through various changes in cardiac thermal limits (T_{ARR}, T_{PEAK}, T_{QB}, T_{AB}), peak f_{Hmax} and f_{Hmax} at common test temperatures (Marchant and Farrell, 2019; Eliason and Anttila, 2017; Fig. 6). These changes are both species-specific and context dependent. For example, at common, moderate test temperatures, cold-acclimated Atlantic salmon (*Salmo salar*) had a higher f_{Hmax}, along with lower thermal limits and peak f_{Hmax} than their warm-acclimated counterparts (Anttila et al. 2014a). While several species display the same classic thermal compensation response of f_{Hmax} – being higher over moderate temperatures after cold acclimation (Anttila et al. 2014a; Adams et al. 2022; Drost et al. 2016b; Gilbert and Farrell, 2021; Fig. 6) – this is not universal. For example, in killifish (*Fundulus heteroclitus*), an acclimation temperature of 15°C produced the highest f_{Hmax} at a common temperature when compared to both cold (5°C) and warm (33°C) acclimation (Safi et al. 2019). Yet, peak f_{Hmax} and the thermal limits still increased with acclimation temperature (Safi et al. 2019). Thus, the method can be used to explore the diversity in cardiac thermal plasticity among fishes.

While cardiac thermal limits generally increase with warm acclimation, there is a 'thermal ceiling' for cardiac plasticity, much like for other measures of acute heat tolerance (i.e. LT50, CT_{MAX}). In fact, acclimation close to this thermal ceiling can even lower thermal limits and reduce peak f_{Hmax} (Adams et al. 2022; Gilbert and Farrell, 2021; Pettinau et al. 2022a; Marchant and Farrell, 2019; Strowbridge et al. 2024; Fig.6C). For instance, Adams et al. (2022) performed the method on rainbow trout acclimated to six temperatures from 15 to 25°C. They found that thermal limits increased with acclimation temperature up to 23°C but that these limits and the peak f_{Hmax} achieved during warming all decreased with a further increase in acclimation temperature to 25°C. The f_{Hmax} test can be rapidly assessed across acclimation temperatures to identify this ceiling for species of conservation concern.

Thermal plasticity is time dependent. In CT_{MAX} acclimation rate trials, for example, CT_{MAX} increases logarithmically with time when moved from cold to warm (Fangue et al. 2014). The same principle applies to cardiac plasticity. By studying f_{Hmax} throughout an acclimation (Gilbert et al. 2022b; Hardison et al. 2023) or during fluctuating temperature treatments (Schwieterman et al. 2022), researchers can (1) assess how rapidly the animals can acclimate, (2) better model performance and thermal limits of species in response to environmentally relevant temperature exposures, and (3) examine mechanisms of heart rate resetting across species and in response to secondary stressors. Examining this time-course has revealed varied results in a few studies. In rainbow trout, only T_{Q10} increased while T_{PEAK} and T_{ARR} were unaffected when acclimation temperature was increased from 10 to 18°C (Gilbert et al. 2022b). In the same study, f_{Hmax} over moderate temperatures was rapidly (~72h) reset to a lower level while peak f_{Hmax} rapidly increased, but then subsided after 2 weeks of acclimation to 18°C. In opaleye (*Girella nigricans*), the fish's thermal limits and peak f_{Hmax} increased when warmed from 12 to 20°C for two weeks, but only after being fed a carnivorous or omnivorous diet (Hardison et al. 2023). When the fish were fed an herbivorous diet, their thermal limits still increased, but their peak f_{Hmax} did not. Notably, differences in f_{Hmax} were related to the fatty acid composition of the heart, which was affected by the fish's diet. Future research should investigate how mechanisms of cardiac pacemaker resetting and autonomic control are influenced by thermal exposure time and extrinsic factors – like diet – to understand the relative contributions of these factors make to changes in f_{Hmax} in wild and farmed fishes. The f_{Hmax} method is a valuable assay for examining these mechanisms more closely across taxa, timescales and environments.

Emerging applications and future directions

Several emerging applications and future directions have the potential to expand the utility of the f_{Hmax} method. Given the high-throughput nature of the method, it can be used to study diversity in the plasticity and drivers of cardiac thermal performance across fish taxa. For instance, mechanisms of cardiac failure may differ among species and life-stages based on the extent to which they rely on coronary circulation for cardiac oxygen supply, or based on the relative composition (e.g., spongy vs. compact) of their myocardium (Ekström et al. 2021; Ekstrom et al. 2023). High quality ECG measurements permit detailed ECG waveform analyses and the application of well-established interpretations of the relationships among waveforms to understand changes in the cardiac cycle that underly changes in heart rate and function (e.g. (Badr et al. 2016). This potential has been largely unexploited so far with the f_{Hmax} method (Pettinau et al. 2022a). Additional or alternative pharmacological interventions can target other specific ion channels or regulatory mechanisms. There is substantial room for growth in this regard in addition to the study of pace making mechanisms by Marchant and Farrell (2019), and on-going studies which use non-selective adrenergic antagonists to study intrinsic f_H and the thermal plasticity of adrenergic sensitivity. Lastly, Doppler echocardiography can be used, instead of ECG electrodes, to record cardiac blood flow holistically (Q as function of SV and f_{Hmax}) and reveal additional information regarding the collapse of cardiac function at high temperatures (Muir et al. 2022; Muir et al. 2021). However, how SV and Q in the f_{Hmax} preparation relates to that which occurs under routine or elevated workloads (e.g. exercise and digestion) has not been established.

Conclusions

In summary, the assessment of f_{Hmax} in pharmacologically stimulated, anaesthetized fish, is an effective high throughput method for the study of cardiac thermal performance during acute warming and the quantification of upper thermal limits. The resulting f_{H} and thermal performance metrics are valuable for the basic study of cardiac function and in an applied context for understanding how rapid, ongoing environmental change may impact fishes. While these metrics are valuable on their own, the method also provides an excellent starting point for studying mechanisms that underly temperature effects on cardiac and cardiorespiratory performance at lower levels of organization (e.g. isolated myocytes, mitochondria, and ion channels) and integrated outcomes at the whole animal level. For instance, subsequent targeted molecular studies or whole-animal performance assessments can be performed at the specific temperatures that were identified as limiting (i.e. T_{AB}, T_{PEAK}, and T_{ARR}) in the f_{Hmax} method without having to generate full thermal performance curves for traits that are far more time consuming to assess. Such studies are more urgent now than ever as a thorough understanding of the causes and consequences of physiological responses to variable thermal regimes will assist in predicting and mitigating outcomes of global environmental change.

Data availability

To assess thermal acclimation responses, relationships between thermal limits and trends across studies, we compiled data from all studies that cited the study first proposing the method (Casselman et al. 2012), and in which animals were held under their treatment temperature conditions for >1 week before testing. The compiled data includes ‘control’ treatments (i.e. no co-occurring stressor effects) and treatments that represent natural sources of variation within a population (i.e. size, life stage, diet). The data include mean values for metrics from the f_{Hmax}

assessment and additional relevant metadata. They are available in the following public repository: https://doi.org/10.6084/m9.figshare.25661178.v1

Tables:

Table 1. Definitions and suggested interpretation for transition temperatures or thermal limits identified through the assessment of maximum heart rate (f_{Hmax}) during acute warming.

Temperature	Definition	Suggested Interpretation
T_{AB}	Arrhenius breakpoint temperature above which the slope of f_{Hmax} vs. temperature on an Arrhenius plot is distinctly limited	Above T_{AB} the increase in f_{Hmax} is generally limited relative to f_{Hrest} resulting in a loss of scope for f_{H}; Commonly, corresponds with optimal or pejus temperatures around peak aerobic capacity (Casselman et al. 2012)
T_{Q10}	Temperature at which the incremental Q_{10} temperature coefficient is limited to values below the selected threshold, commonly 1.9 or 2.0 for the remainder of the acute warming challenge.	Similar to $T_{AB,}$ above $T_{Q10,}$ the increase in f_{Hmax} with further warming is limited relative to what is typical for f_{Hrest} and routine oxygen which can in turn constrain the f_{H} and aerobic scope for vital functions (Fig. 1).
T_{PEAK}	Temperature at peak f_{Hmax} during warming	f_{Hmax} cannot increase any further and so f_{H} scope and thus aerobic scope become critically limited as f_{Hrest} rises while f_{Hmax} does not (Fig.1). Whole-animal performance is vulnerable to oxygen limitation under elevated aerobic workloads.
T_{ARR}	Temperature at the onset of cardiac arrhythmia	f_{H} cannot be sustained and cardiac collapse has occurred or is imminent. Whole-animal performance and survival are time-limited in agreement with the common proximity to CT_{MAX} (Fig. 4)

Figure captions:

Figure 1. The effect of temperature on A) maximum and resting heart rate (f_{H}; solid lines) and oxygen uptake (M□O_2; dashed lines) with B) the resulting changes in f_{H} and aerobic scope in adult sockeye salmon (*Oncorhynchus nerka)*. Equations are adapted from (Eliason et al. 2013).

Figure 2. A common laboratory configuration for the assessment of f_{Hmax} during acute warming (A) with representative ECG recordings (B-D) and resulting data (E-G). ECG recordings are from large (3.2kg) anadromous Arctic char (*Salvelinus alpinus*) at 15°C (B), $\mathrm{T}_{\mathrm{PEAK}}$ (C), and $\mathrm{T}_{\mathrm{ARR}}$ (D) and were recorded in a remote Arctic field setting (Gilbert et al. 2022a). The mean (dark blue connected points, shaded area is SEM) f_{Hmax} response to acute warming (E) is shown with representative individual data (light blue lines; n=8 for display purposes) from the same study, along with the resulting Arrhenius breakpoint (F) and incremental Q_{10} (G) analysis. Labeled arrows indicate the mean thermal limits and peak f_{Hmax}.

Figure 3. Heart rates (f_{H}; mean ±SEM) during acute warming in anaesthetized (tricaine methanesulfonate; TMS) or non-anaesthetized (No TMS) rainbow trout (*Oncorhynchus mykiss*; A) and Arctic char (*Salvelinus alpinus;* B) with or without treatment with atropine and isoproterenol. The mean (±SEM) peak f_{H} achieved during acute warming are shown with grey background. Data for non-anaesthetized rainbow trout are from Gilbert et al. 2019, with data for anaesthetized rainbow trout (unpublished data, M. Gilbert) collected on the same cohort of fish during the same time frame (n=6). Data for captive saltwater acclimated non-anaesthetized and wild upriver-migrating anaesthetized Arctic char are from Penney et al. (2014) and Gilbert et al. (2020) respectively. Arctic char from these two studies are presumed to have similar acclimation temperatures (~10°C; Gilbert et al. 2020 examined wild fish so the precise acclimation temperature was unknown), were of similar size, had an identical peak f_{H} during acute warming (115bpm) and had an identical critical thermal maximum (23°C). The horizontal line indicates temperatures during acute warming at which f_{Hmax} in anaesthetized fish was significantly greater ($p<0.05$; holm adjust pairwise t-tests) than routine f_{H} in non-anaesthetized fish indicating a positive scope for heart rate, which deteriorates at high temperatures as the responses converged.

Figure 4. Data in the figure is from studies compiled as part of the data synthesis (see Data Availability). Panel A shows a plot of the various thermal limits calculated in the f_{Hmax} test in relation to $\mathrm{CT_{MAX}}$. Color indicates the thermal limit, with individual points indicating means from various studies and solid line indicating the line of best fit. Dashed line indicates a 1:1 relationship. B) Shows the relationship between $\mathrm{T_{PEAK}}$ and $\mathrm{T_{ARR}}$ and C) shows $\mathrm{T_{AB}}$ and $\mathrm{T_{Q10.}}$ Simple best fit lines are added along with the equation and fit. Each point is from a different mean value. The dashed line indicates 1:1.

Figure 5. The differences between and progression of thermal limits identified from the assessment of maximum heart rate (f_{Hmax}) during acute warming in anaesthetized fish. Differences are within individual treatments in a study and are shown for all data included in the the data synthesis (A). The progression of thermal limits (B) in a treatment group are shown for the subset of studies in which $\mathrm{T_{Q10}}$, $\mathrm{T_{PEAK}}$, $\mathrm{T_{ARR}}$ and $\mathrm{CT_{MAX}}$ were all available. In both plots, colors indicate the acclimation temperature. See ‘Data Availability’.

Figure 6. Plasticity in the response of maximum heart rate (f_{Hmax}) to acute warming and associated change in cardiac thermal limits. The f_{Hmax} (A) and resulting incremental Q_{10} values (B) during acute warming are shown (mean ±SEM) for lab reared Arctic char (472g; *Salvelinus alpinus)* acclimated to 2,6,10 and 14°C (modified from Gilbert and Farrell 2021). The arrows (A and B) highlight the thermal compensation of f_{Hmax} and an increase in peak f_{Hmax} and cardiac thermal limits, which are common (but not universal) aspects of cardiac thermal plasticity. The corresponding thermal limits including temperatures at the Q_{10} threshold ($\mathrm{T_{Q10}}$), peak f_{Hmax} ($\mathrm{T_{PEAK}}$), and the onset of cardiac arrhythmia ($\mathrm{T_{ARR}}$) as well as the critical thermal maximum ($\mathrm{CT_{max}}$) are shown (C) including for fish acclimated to 18°C, a temperature at which mortality was elevated and feeding had ceased. The same thermal limits (excluding $\mathrm{CT_{max}}$) and the Arrhenius breakpoint temperatures ($\mathrm{T_{AB}}$) are shown (D) for all studies examined in our data synthesis (see ‘data availability’), with simple lines of best fit (± 95% confidence intervals) for each thermal limit over acclimation temperature (R^2 $\mathrm{T_{ARR}}$: 0.57, $\mathrm{T_{PEAK}}$: 0.66, $\mathrm{T_{Q10}}$: 0.68, $\mathrm{T_{AB}}$: 0.58). Error bars or shading are encompassed by the symbol or line if not visible.

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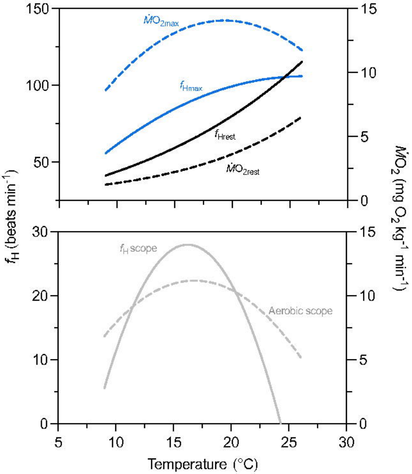
150
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0
$\dot{M}O_{2max}$
f_{Hmax}
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f_H (beats min^{-1})
$\dot{M}O_2$ (mg O_2 kg^{-1} min^{-1})
30
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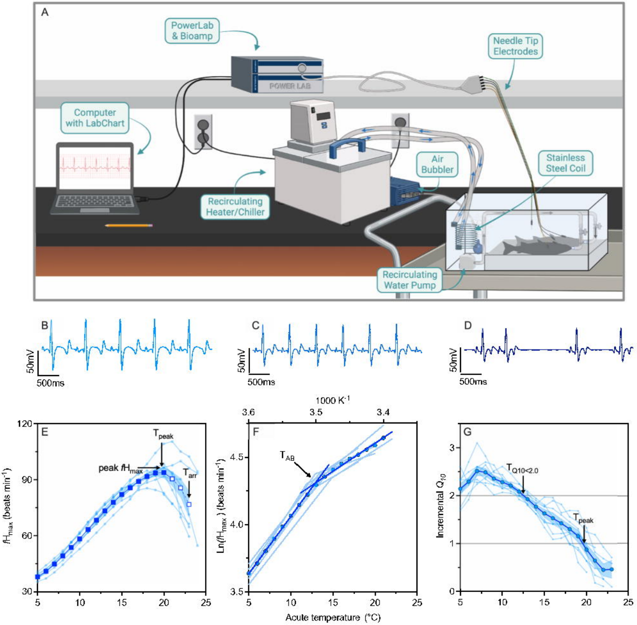
30
Temperature (°C)

Figure 1


A
PowerLab & Bioamp
POWER LAB
Needle Tip Electrodes
Computer with LabChart
Air Bubbler
Stainless Steel Coil
Recirculating Heater/Chiller
Recirculating Water Pump
B
50mV
500ms
C
50mV
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D
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E
fH_{max} (beats min^{-1})
120
90
60
30
5
10
15
20
25
T_{peak}
peak fH_{max}
T_{arr}
F
1000 K^{-1}
3.6
3.5
3.4
Ln(fH_{max}) (beats min^{-1})
4.5
4.0
3.5
T_{AB}
5
10
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20
25
Acute temperature (°C)
G
Incremental Q_{10}
3
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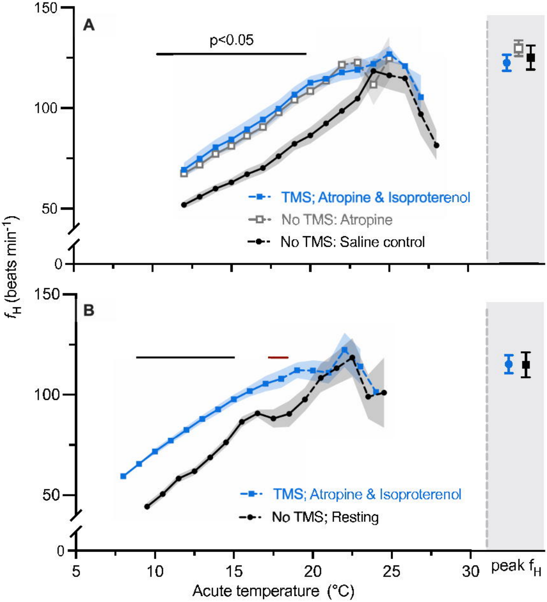
Figure 2

A
B
p<0.05
TMS; Atropine & Isoproterenol
No TMS: Atropine
No TMS: Saline control
TMS; Atropine & Isoproterenol
No TMS; Resting
150
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f_H (beats min^{-1})
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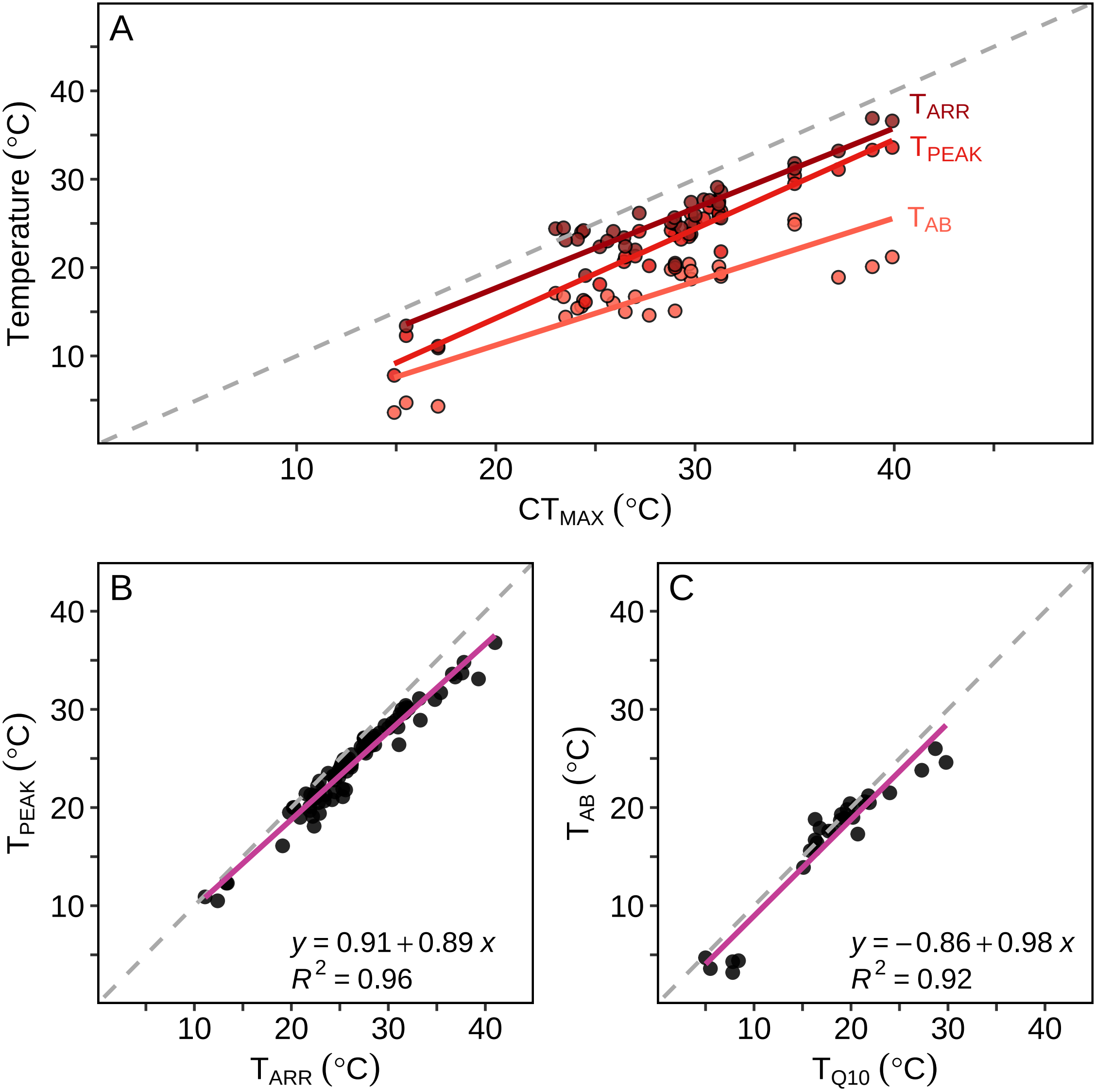
peak f_H
Acute temperature (°C)

Figure 3


A
Temperature (°C)
10
20
30
40
T_{ARR}
T_{PEAK}
T_{AB}
10
20
30
40
CT_{MAX} (°C)
B
T_{PEAK} (°C)
10
20
30
40
$y = 0.91 + 0.89\,x$
$R^2 = 0.96$
10
20
30
40
T_{ARR} (°C)
C
T_{AB} (°C)
10
20
30
40
$y = -0.86 + 0.98\,x$
$R^2 = 0.92$
10
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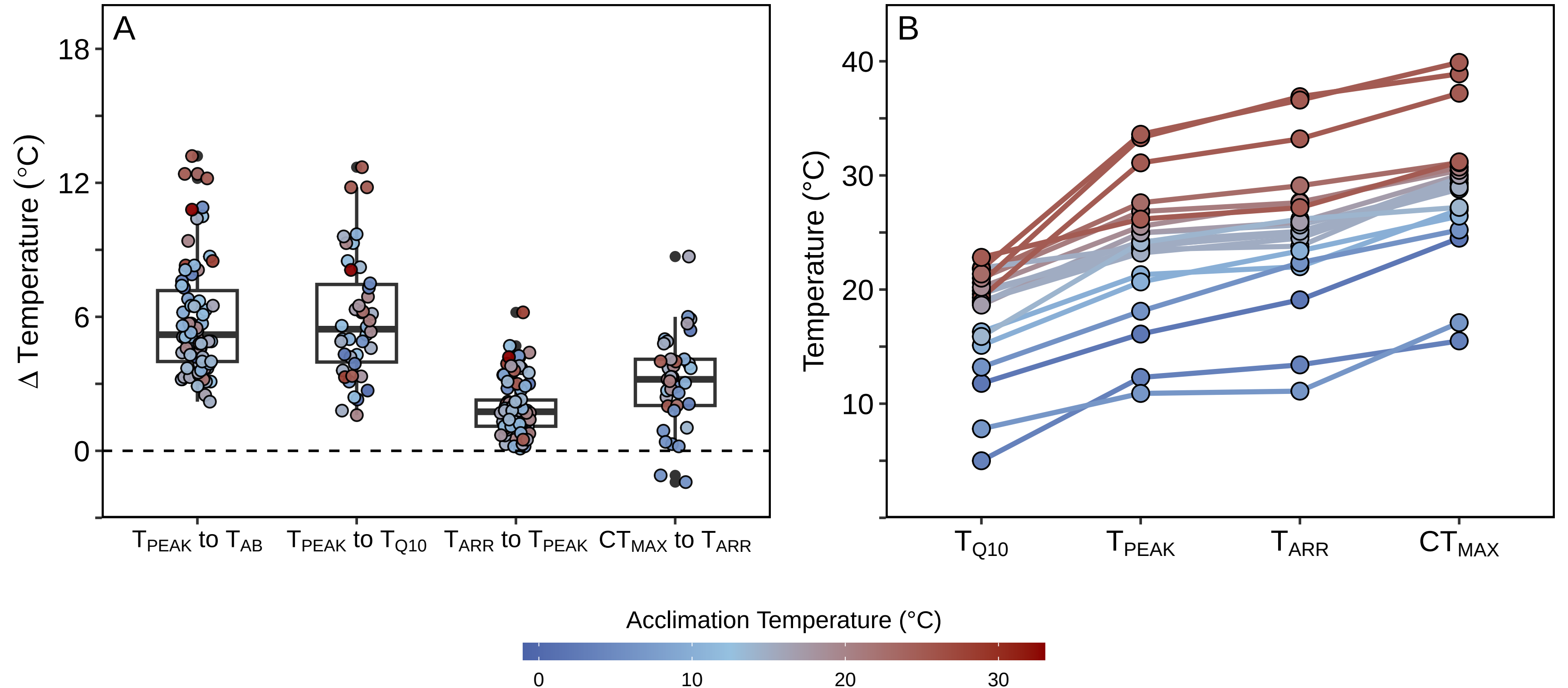
40
T_{Q10} (°C)


A
Δ Temperature (°C)
18
12
6
0
TPEAK to TAB
TPEAK to TQ10
TARR to TPEAK
CTMAX to TARR
B
Temperature (°C)
40
30
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10
TQ10
TPEAK
TARR
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Acclimation Temperature (°C)
0
10
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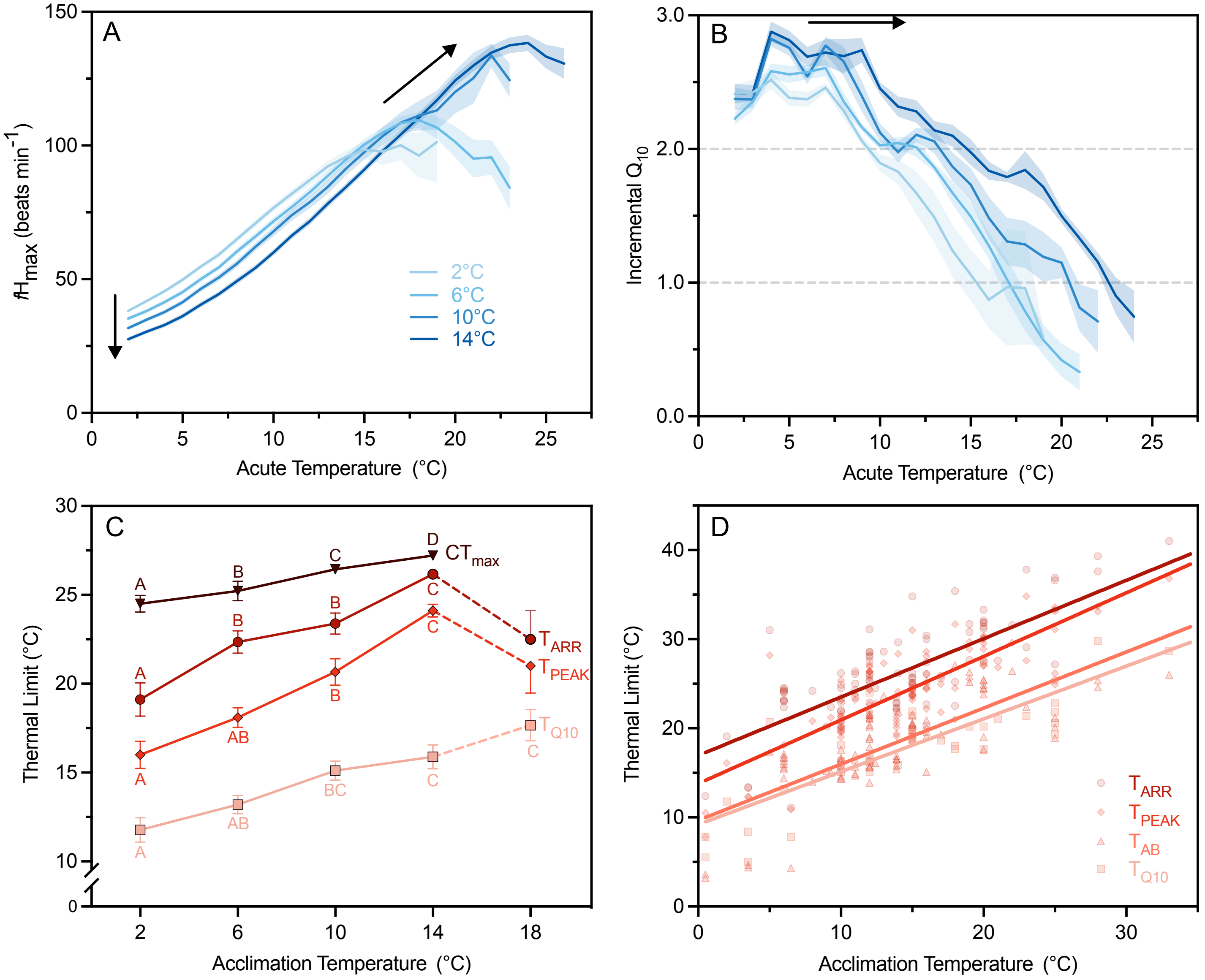
A
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D
fH_{max} (beats min^{-1})
Acute Temperature (°C)
2°C
6°C
10°C
14°C
Incremental Q_{10}
Thermal Limit (°C)
Acclimation Temperature (°C)
CT_{max}
T_{ARR}
T_{PEAK}
T_{Q10}
T_{AB}
0
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15
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Figure 6