Does tolerance to fast warming predict tolerance to slow warming in fishes?

Authors

Lechner, E.R.[1*], Stewart, E.M.C.[2*], Berry, A.A.[3], Bihun, C.J.[2], Binning, S.A.[4], Blewett, T.A.[5], Cadonic, I.G.[5], Clark, T.D.[3], Cowan, Z.-L.[1], De Bonville, J.[6], Ern, R.[7], Gradito, M.[3], Green, L.[1,8], Hoots, E.C.[3], Kuchenmüller, L.L.[3], Mélançon, V.[4], Metz, M.[7], Pfeufer, L.[1], Pottier, P.[1], Raby, G.D.[2,6], Roche, D.G.[9], Sundin, J.[10], Tamarit-Castro, E.[11], Tomás, M.[1], Vigneault, J.[12], Zouhair, Z.[13], Åsheim, E.R.[10] and Jutfelt, F.[1]

[1] Department of Biological and Environmental Sciences, Faculty of Science, University of Gothenburg, Gothenburg, 413 90, Sweden
[2] Environmental and Life Sciences, Trent University, Peterborough, Ontario, K9J 1Z8, Canada
[3] School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, 3216, Australia
[4] Département de Sciences Biologiques, Université de Montréal, Montréal, H3T 1J4, Canada
[5] Department of Biological Sciences, Faculty of Science, University of Alberta, Edmonton, T6G 2E9, Canada
[6] Department of Biology, Trent University, Peterborough, Ontario, K9L 0G2, Canada
[7] Department of Biology, Faculty of Natural Sciences, Norwegian University of Science and Technology, Trondheim, 7491, Norway
[8] Maritime Museum & Aquarium, Gothenburg, 414 59, Sweden
[9] Institut de Biologie, Université de Neuchâtel, Neuchâtel, 2000, Switzerland
[10] Department of Aquatic Resources, Swedish University of Agricultural Sciences, 178 93 Drottningholm, Sweden
[11] Department of Earth Sciences, Faculty of Science, University of Gothenburg, Gothenburg, 413 90, Sweden
[12] Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski, Rimouski, G5L 3A1, Canada
[13] Department of Marine Sciences, Faculty of Science, University of Gothenburg, Gothenburg, 413 90, Sweden

***corresponding authors/leading authors:** Emily Lechner at emily.lechner@bioenv.gu.se University of Gothenburg | Erin Stewart at erinstewart@trentu.ca Trent University

Abstract

1. Critical thermal maximum (CT_{max}) is widely used to quantify upper thermal limits in fishes, but its ecological relevance has been questioned because standard protocols use fast warming rates (0.3°C min^{-1}; 18°C h^{-1}) that rarely occur in nature, where thermal stress more typically unfolds over hours to days. Whether CT_{max} under fast warming predicts warming tolerance to slower, ecologically realistic warming remains a critical question for climate risk assessments and mechanistic species distribution models.
2. We measured thermal tolerance under fast (0.3°C min^{-1}) and slow (1°C h^{-1}) warming in the same individuals across seven temperate fish species. This paired design allowed us to estimate individual-level correlations between fast and slow warming tolerance and assess how this relationship varied among species.
3. We observed substantial interspecific variation in the direction of the fast–slow warming tolerance relationship. Slow CT_{max} was higher than fast CT_{max} in three-spined stickleback, lesser pipefish, corkwing wrasse, and European eel, while the opposite was true for European plaice, bluegill sunfish, and Atlantic cod.
4. Fast and slow CT_{max} were only weakly correlated at the individual level, and significant correlations were only detected in two of seven species. However, at the species level, fast and slow CT_{max} was significantly correlated (R^2 marginal = 0.873; between-species slope = 1.157).
5. Standard fast-warming CT_{max} does not consistently predict individual thermal tolerance under slower, more ecologically realistic warming rates and its usage may overlook biologically meaningful species-specific differences in thermal vulnerability. Fast-warming CT_{max} should be interpreted with caution in the context of ecological forecasting and climate risk assessments as it can over-estimate upper thermal tolerance and should be used at the appropriate biological scale.

Keywords

Climate change, ectotherms, ramping rate, heat stress, thermal ecology, warming tolerance, heating rate, Sweden

Introduction

As the climate warms, scientists and policymakers seek to understand the impacts of environmental change on animal physiology. A key challenge is to develop tools that can predict species responses to climate warming to enable accurate forecasting and proactive management. For ectotherms, a widespread tool is the critical thermal maximum (CT_{max}) test, which consists of acute warming – typically at 0.3°C min^{-1} – until a temperature is reached where the animal experiences loss of equilibrium (LOE) (Cowles and Bogert, 1944; Desforges et al., 2023; Ern et al., 2023; Raby et al., 2025a). The CT_{max} method has several advantages over other protocols for estimating thermal tolerance, including being largely non-lethal and relatively quick and simple (Beitinger and Lutterschmidt, 2011; Raby et al., 2025a). Additionally, CT_{max} is often a repeatable trait within individuals (Morgan et al. 2018; Chasse et al., 2025; Grinder et al., 2020). Moreover, CT_{max} can correlate with global distribution patterns (Sunday et al., 2019), suggesting it is a good proxy of the thermal niche occupied by organisms in nature. Therefore, use of the CT_{max} method is increasing rapidly, and it is being applied in

meta-analyses and species distribution modelling (Raby et al., 2025a; Pottier et al., 2025; Molina et al., 2025).

The rapid temperature change experienced during a CT_{max} trial (usually 0.3°C min^{-1}) may be applicable to some natural warming scenarios, such as for animals moving rapidly through thermal heterogenous environments, and those living in extreme environments such as tidal pools where rapid temperature spikes can take place (see review in Desforges et al., 2023). CT_{max} is also a valuable tool for comparative and mechanistic studies, such as testing for interacting stressors and thermal acclimation dynamics (Raby et al., 2025b; De Bonville et al., 2025). However, it remains unclear, how well tolerance to fast warming predicts tolerance to slower warming, which is more typical in most aquatic environments and is more reflective of natural heat waves (Desforges et al., 2023; Iacarella et al., 2025; Åsheim et al., 2020; Ern et al., 2023).

Attempts to link fast-warming CT_{max} to tolerance of prolonged heat exposure have yielded mixed results. In 25−600 g Atlantic salmon (*Salmo salar*), CT_{max} measured under a very fast warming rate (0.4°C min^{-1}) did not correlate with chronic thermal tolerance measured under a slow warming rate (incremental temperature warming with diel fluctuations [ITD_{max}] variations of ~0.25°C day^{-1}; Bartlett et al., 2022). This mismatch possibly occurred because the warming rate was too rapid to allow internal thermal equilibrium (Raby et al., 2025a), which may contribute to an overestimation of CT_{max} in large fish. In contrast, a study of individually tagged zebrafish (*Danio rerio*) found a significant correlation between fast (0.3°C min^{-1}) and slow (0.025°C min^{-1}) warming tolerance in cold-acclimated fish, with a weaker relationship in warm-acclimated individuals (Åsheim et al., 2020). A recent study in juvenile coho salmon (*Oncorhynchus kisutch*) found that fast-warming CT_{max} estimates can be 2–3°C higher than those obtained during very slow warming (ITD_{max}; Iacarella et al., 2025). While these findings suggest fast CT_{max} may predict tolerance to slower warming under certain conditions, the generality of this trend across species remains uncertain (Åsheim et al., 2020; Raby et al., 2025a).

Differences between estimates of thermal tolerance obtained under fast and slow warming rates are predicted to occur due to multiple reasons. Exposure to slower warming can lead to a higher accumulation of thermal injury per degree of warming, resulting in a lower CT_{max} (Ern et al., 2023; Ørsted et al., 2022; Rezende et al., 2014) than during fast warming. Consistent with this idea, comparisons across fish species have shown that warming rate can strongly influence measured thermal tolerance, with lower CT_{max} often observed under sufficiently slow warming rates because of prolonged exposure to damaging temperatures (Mora and Maya, 2006). Thermal acclimation, defined as physiological adjustments in response to changes in temperature (Somero et al., 2016; Seebacher et al., 2015; Jutfelt et al., 2024), can also confound the comparison of estimates obtained under different ramping rates. Recent evidence suggests that some fishes show a noticeable acclimation response within a few hours of experiencing a temperature change (De Bonville et al., 2025; Metz et al., 2025; Serres et al., 2026), whereas in others it can occur after a day (Stewart et al., 2023). Slow warming rates may thus allow fish with a rapid acclimation response to increase their thermal tolerance within a trial, whereas fast-warming protocols are likely too fast to allow acclimation responses to have an effect. Overestimates of thermal limits induced by rapid acclimation may be especially pronounced when slow

warming rates are started at low temperatures, since the capacity for increasing CT_{max} through acclimation is higher when organisms are cold, rather than warm acclimated. This is likely due to a physiological ceiling to the plasticity of warming tolerance limits (Morgan et al., 2022; Ern et al., 2023; Fry 1971; Brett 1956). There are also large differences in acclimation dynamics and capacity between teleost species (Tamarit-Castro et al., 2026). Therefore, the difference between measurements of CT_{max} under slow and fast warming likely depends on the species as well as the dynamics of thermal acclimation and injury accumulation.

Here, thermal tolerance was measured under fast warming and subsequently slower warming across seven teleost species from both freshwater and marine environments. We measured CT_{max} in the same individuals under fast warming (0.3°C min^{-1}) and slow warming (0.017°C min^{-1}; 1°C h^{-1}) to quantify the intra-individual correlation between estimates obtained under these two methods. Similarly to results obtained in zebrafish (Åsheim et al., 2020), we expected a positive correlation between thermal tolerance measured under fast and slow warming rates at the individual level. We also aimed to reveal how different warming rates affect estimates of upper thermal tolerance, which is important for determining the utility of the vast CT_{max} literature for climate risk assessments and species distribution models.

Materials and Methods

Animal research ethics

Fish were collected and studied under ethical permits 5.8.18-8955/2022 and 5.8.18-07281/2025 issued to F. J., approved by the Swedish Board of Agriculture's ethical committee. European eel (*Anguilla anguilla*) were collected and released under fisheries regulations exempt Dnr SLU.aqua.2025.5.2-50, issued by the Swedish University of Agricultural Sciences, in accordance with decision Dnr 000031-2025 by the Swedish Agency for Marine and Water Management, and in accordance with permit Dnr: 28731-2025, issued by the County Administrative Board of Västra Götaland to J. S. Bluegill sunfish (*Lepomis macrochirus*) were collected and studied under the scientific collection permit PEBA-2025-FWCA- 00322 and animal use protocol 29711 issued to G. R. (issued by the Trent University Animal Care Committee, and following guidelines set by the Canadian Council on Animal Care).

Study species and collection

Experiments were conducted on four marine fish species (Atlantic cod [*Gadus morhua*], corkwing wrasse [*Symphodus melops*], European plaice [*Pleuronectes platessa*], lesser pipefish [*Syngnathus rostellatus*]), one brackish water species (three-spined stickleback [*Gasterosteus aculeatus*]), one diadromous species (European eel [*Anguilla anguilla*]) and one freshwater species (bluegill sunfish [*Lepomis macrochirus*]). Individuals of both European eel and European plaice were tested as juveniles, all other species in the experiment were adults. Marine, brackish and diadromous species were collected from April 26–June 22, 2025, and studied at the Kristineberg Marine Research Station (58.2498° N, 11.4458° E), Sweden, during June 8–16, 2025 (for Atlantic cod) and June 18–July 3, 2025 (for remaining marine/brackish/diadromous species) (Table S1, Table 1). Bluegill sunfish were collected from the Otonabee river (44.3744°N, -78.2852°W), and studied at Trent University, Ontario,

Canada (44.3547° N, 78.2871° W), from September 8–11 (collection) and November 9–14 (studied), 2025 (Table S1, Table 1).

For the experiments at Kristineberg, fishes were collected near the marine station using beach seine, baited traps and fyke nets (Table S1). All fishes were immediately transported to the research station and acclimated in flow-through tanks with species-appropriate habitat structure (sand, algae, PVC tubes, or plants) and photoperiod regimes that matched seasonal conditions (Table S1). Three-spined sticklebacks — although considered brackish — were collected in marine waters within 1 km from the station (58.2428°N, 11.4683°E) whereas European eels were collected in elver traps by the mouth of river Viskan, on the west-coast of Sweden (57.1337°N, 12.1235°E). Acclimation temperatures approximated ambient conditions at collection sites (16°C for most marine/brackish/diadromous species collected in Sweden in June–July; ~9°C for Atlantic cod; 21°C for bluegill sunfish). Fishes were fed to satiation with species-specific diets prior to trials and fasted for ~24–48 hours before CT_{max} testing (Table S1).

Experimental protocol

CT_{max} trials were conducted as a series of replicate trial groups for each species (Figure S1). Replicates were used to reduce the number of individuals to observe within a given trial, to make individual identification possible, and to account for trial effects (Raby et al., 2025a). For most species, four replicate trial groups were used: individuals remained with their trial group throughout the experimental period, during housing and in both CT_{max} trials, with approximately similar numbers of individuals in each trial group within a species (Table 1). Fast-warming trials were always conducted before slow-warming trials due to a higher expected mortality following slow-warming trials (Åsheim et al., 2020). Slow-warming trials commenced three days (five for European eel, and between six and eight for Atlantic cod) after the fast-warming trials to allow for recovery (Table 1).

The same observers were used to evaluate CT_{max} endpoints for fast and slow trials of a given species to ensure similar endpoint determination. Endpoints were species-specific and were defined as loss of equilibrium (LOE), non-responsiveness, or ventilatory arrest; the latter two endpoints were applicable to the European eel, while the first two were applicable for the three-spined stickleback, and all other species were warmed until LOE (Table 1). European eels express different behaviours during warming than other fishes, as seen in Belaud et al. (1979) where spontaneous interruptions in the eel's ventilatory activity occurred. These interruptions in the eel's ventilatory activity were also observed in our study. For the three-spined stickleback, while some individuals exhibited a loss of equilibrium while swimming as seen in other species, others went stiff and non-responsive in response to warming, which we recognized as a second, alternative endpoint classification for this species. Observers were blinded to arena temperatures during trials and removed individuals as soon as their behavioural endpoint was reached. The time (to the nearest second), temperature (°C), and endpoint type were recorded for each individual when an endpoint was observed. Fish were moved to individual containers (at acclimation temperature, 9, 16, 21°C) upon removal from the arena to monitor recovery and uniquely identify them for correlation analyses. For Atlantic cod, a FireSting-PRO (Pyro Science, Aachen, Germany) with fiber-optic oxygen sensor and a temperature sensor made continuous measurements and data were manually logged for calculating the warming rate. Recovery was recorded 0.5 and 2 h following the

trials for all species, except bluegill sunfish, which were checked only after 0.5 h. Recovery containers were refreshed with ambient water periodically and aerated throughout the recovery period.

Fish were identified using either body length and mass (Atlantic cod) or by using visible implant elastomers. Once fish had recovered from the fast trial, the total length and mass of each individual was recorded. Sex was not determined, other than with the gravid males in lesser pipefish. Visible implant elastomers (Northwest Marine technologies, USA) were injected into the dorsal musculature under light anesthesia using 0.125 g L^{-1} tricaine methanesulfonate (MS222) (0.18 g $L^{-1}$for eel), in which each individual was given a unique colour code (two lines).

When the slow trials were completed, fish that recovered (after the 2h recovery period) were released near Kristineberg in accordance with the ethical permit. For bluegill sunfish, which were collected and studied in Canada, they were euthanized following experiments.

Fast-warming trials

Fast-warming trials were conducted at a rate of ~0.3°C min^{-1} following the methodology described in Morgan et al. (2018) and Raby et al. (2025a) (see Metadata on FigShare). Described briefly, fish were placed in a tank divided into two compartments by a mesh screen: 1) a heating compartment with a heater element and a small pump providing water flow over the heater and out of the heating compartment, and 2) an arena compartment holding the fish. Fish were acclimated for 15–30 min in the arena at their holding temperature before warming began (trial-specific warming rates in Figures S1, S3). Temperature was continuously monitored during trials with high-accuracy probes (Testo 112 https://www.testo.com/en-, Testo Ltd, Hampshire, United Kingdom; YSI Prosolo, YSI inc., USA; Firesting GO_2 pocket oxygen meter and FireSting-PRO, Pyro Science, Aachen, Germany; PT100, ThermoWorks, USA).

Slow thermal warming trials

Slow warming trials were conducted at a rate of ~1°C h^{-1} using either 1) temperature-controlled sumps supplying multiple test arenas or 2) manual adjustments of heating power (Figures S2, S3; see Metadata on FigShare). For 1), the system consisted of a temperature-controlled sump that supplied two paired CT_{max} arenas via Eheim Universal pumps (Eheim 600; 10W; Deizisau; Germany), and the water returned to the sump via an overflow. To maintain high water quality, the sump was continuously flushed with fresh seawater at 0.1–0.2 L min^{-1}. To control the warming rate, a programmable thermostat system was used (SmartPID, two-channel system), connected to two titanium heating rods (500W + 300W, Aquamedic, Germany; see Metadata on FigShare for specifics for each species). This set-up allowed controlled warming with precise control of the sump temperature. Warming rates were logged with RBR Solo^3 loggers (https://rbr-global.com/) or HOBO pendants (https://www.onsetcomp.com/) within the test arena. For system 1), fish were acclimated for 30-60 min. For system 2), which was was used for both Atlantic cod and bluegill sunfish, a larger CT_{max} arena was used and continuously fed with heated water from sumps, and warming rate was maintained via adding or removing heaters (titanium or glass rod heaters) within the sumps. Two European eel slow CT_{max} trials were removed from analysis due to an irregular warming rate near the end of the trials caused by equipment malfunction.

The mean warming rates for fast and slow trials by species were: Atlantic cod 0.29°C min^{-1}, 0.97°C h^{-1}; bluegill sunfish 0.28°C min^{-1}, 1.0°C h^{-1}; corkwing wrasse 0.29°C min^{-1}, 0.85°C h^{-1}; European eel 0.30°C min^{-1}, 0.88°C h^{-1}; European plaice 0.29°C min^{-1}, 0.80°C h^{-1}; lesser pipefish 0.31°C min^{-1}, 0.98°C h^{-1}; three-spined stickleback 0.32°C min^{-1}, 0.87°C h^{-1} (Figures S1-S3).

Table 1. Fast and slow CT_{max} trial information for all study species.

Species	Arena	Type	Trial Dates (2025)	# Trials	Individuals / Trial	Survival (%)	Endpoint Type
Atlantic cod (*Gadus morhua*)	32 L + 10 L water bath	**Fast**	June 8–9	4	2–5	100	LOE
	see Metadata	**Slow**	June 15 & 16	4	2–5	73	
Bluegill sunfish (*Lepomis macrochirus*)	75 L + 15 L water bath	**Fast**	Oct 9–11	5	8–10	100	LOE
	see Metadata	**Slow**	Oct 12–14	3	10–17	79	
Corkwing wrasse (*Symphodus melops*)	~23 L	**Fast**	June 22–23	4	15	100 (11 died overnight)	LOE
	see Metadata	**Slow**	June 26–27	4	6–15	96	
European eel (*Anguilla anguilla*)	~14 L	**Fast**	June 27	4	7–10	100 (recovery post-tagging 87.5%)	1) LOE 2) Non-responsive 3) Stopped breathing
	see Metadata	**Slow**	July 2	2	7–10	91 (released July 11)	
European plaice (*Pleuronectes platessa*)	~9 L	**Fast**	June 18–19	5	19–20	Trial 2: 70%; Trials 1, 3–5: 100%	LOE
	see Metadata	**Slow**	June 22–23	5	9–20	94	
Lesser pipefish (*Syngnathus rostellatus*)	~14 L	**Fast**	June 24	4	14–21	87	LOE
	see Metadata	**Slow**	June 27	4	8–18	96	
Three-spined stickleback (*Gasterosteus aculeatus*)	~14 L	**Fast**	June 26	4	14–15	96 (11 died post-tagging)	1) LOE 2) Non-responsive to touch
	see Metadata	**Slow**	June 29	4	6–12	100	

*The five bluegill sunfish fast trial replicates were combined into three slow trial replicates (see *Experimental Protocol*).

Statistical Analysis

Statistical analyses were conducted in R version 4.5.3 (2026.03.11) (R Core Team, 2024).

Effects on CT_{max}

For each species and at both warming rates a single linear mixed effects model (LMM) was used to assess the fixed effects of body size (mass, g), endpoint type (for applicable species; i.e., European eel and three-spined stickleback), reproductive stage (gravid or non-gravid individuals for lesser pipefish

due to visible brood pouch), and recovery (mortality post-trial) on CT_{max}, with trial replicate included as a random effect in each model (seven species, two warming rates; 14 models total). We used 'lmer' in the *lme4* package to fit LMMs (Bates et al., 2015) and used the same covariate structure for the fast and slow model within each species, other than in cases where a factor was only applicable to one warming rate (e.g., if mortalities only occurred in fast trials). Individual ID was not included as a random effect given fish were only sampled once per warming rate; thus, the data were independent within each warming rate. We inspected the estimated variance component for the random effect and assessed model singularity. Although the random effect for trial showed a singular fit in some models, removal of this term resulted in poorer residual diagnostics, so it was retained to maintain appropriate error structure and consistency among models. Model fit and assumptions were checked through visual inspection of residuals and the 'check_model' function in the *performance* package (Lüdecke et al., 2021). Model R^2 (marginal and conditional) was calculated using the 'r2' function and the intraclass correlation coefficient (ICC; adjusted and conditional) of the random effect (trial) was calculated using the 'icc' function, both in the *performance* package (Nakagawa's R^2 for LMMs in Nakagawa and Schielzeth, 2013; Lüdecke et al., 2021).

Fast-slow correlation

Only fish that recovered from the fast trial and could be uniquely identified following both the fast and slow trials were included in the correlation analysis (correlation sample sizes: Atlantic cod $n = 11$, bluegill $n = 34$, corkwing wrasse $n = 47$, European eel $n = 15$, European plaice $n = 81$, lesser pipefish $n = 50$, three-spined stickleback $n = 34$). Therefore, the correlation between fast and slow CT_{max} was analyzed using the residuals of the mixed effects models, which allowed us to control for mass, trial, and other fixed effects in our assessment of whether relative performance at a fast warming rate can predict relative performance at a slow warming rate, as the residuals represent individuals after accounting for covariates. Although some model residuals were normally distributed, some model residuals were left skewed, violating an assumption of Pearson's correlation. Spearman's rank correlation, which assesses monotonic associations without assuming linearity or normality, was therefore used consistently across all species to ensure comparability. Spearman's rank correlation is also robust to tied values and less sensitive to outliers and scale differences. It should be noted that this approach treats residuals as observed data, which influences the confidence intervals on rho (ρ). As a result, we did not run additional cross-species comparisons on the correlations.

Species-level differences

For each species, the mean difference (ΔCT_{max}, °C; slow − fast) was calculated along with corresponding 95% confidence intervals (CI). To evaluate whether body mass influenced ΔCT_{max}, linear models including species with and without body mass were compared using Akaike's Information Criterion (AIC) (Figure S4). Inclusion of body mass did not improve model fit ($\Delta AIC < 2$) and was therefore not retained in subsequent analyses.

To assess whether fast CT_{max} predicts slow CT_{max}, we fitted a linear mixed model (LMM) using individual paired CT_{max} values (n = 272) with slow CT_{max} as the response. Fast CT_{max} was decomposed into a within-species component (individual deviation from the species mean) and a between-species

component (species mean), entered as separate fixed effects, allowing independent estimation of within- and between-species slopes. Species identity was included as a random intercept. The model was fitted by REML using the bobyqa optimizer (*lme4*; Bates et al., 2015). Variance explained was quantified using marginal R^2 (fixed effects only) and conditional R^2 (fixed + random effects; Nakagawa and Schielzeth, 2013, via the *performance* package).

Results

Species and individual-level differences

The within-individual difference in CT_{max} (ΔCT_{max} = slow − fast) varied among species in both magnitude and direction, ranging from −1.64°C (95% CI: -2.09, -1.18) in Atlantic cod to +1.77°C (95% CI: 1.45, 2.1) in European eel (Figure 1, Figure 2, Table S2). Four species (three-spined stickleback, lesser pipefish, corkwing wrasse, and European eel) exhibited higher CT_{max} under slow warming, whereas three species (European plaice, bluegill sunfish, and Atlantic cod) exhibited higher CT_{max} under fast warming (Figure 2). Confidence intervals for most species did not overlap zero, indicating consistent differences between warming rates within species (overall ΔCT_{max} mean 0.31°C, 95% CI: 0.2, 0.43). Within species, correlation between CT_{max} under fast and slow warming rates was variable but generally positive, with significant correlations in bluegill sunfish (r = 0.45 [95% CI: 0.13, 0.68], p = 0.008) and three-spined stickleback (r = 0.42 [95% CI: 0.10, 0.66], p = 0.014), while other species exhibited weak or non-significant associations between fast and slow warming CT_{max} (Figure 3, Table S3).

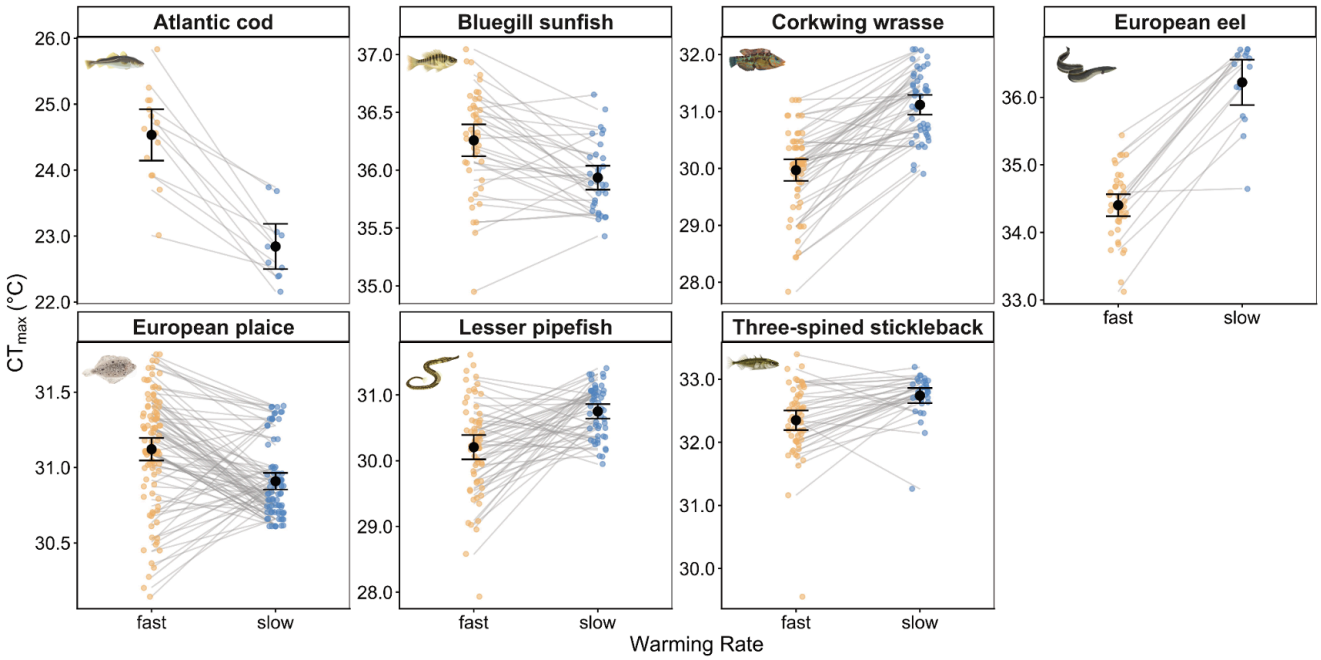

Figure 1. CT_{max} (°C) of the seven study species under fast (18°C h^{-1}; 0.3°C min^{-1}) and slow (1°C h^{-1}) warming rates. Black circles represent the mean and error bars represent the 95% confidence interval. Each coloured point represents an individual fish, and light grey lines join an individual's paired fast and slow warming CT_{max} values, if it recovered from both trials. Note the differing y-axis range among study species and that none start at 0.

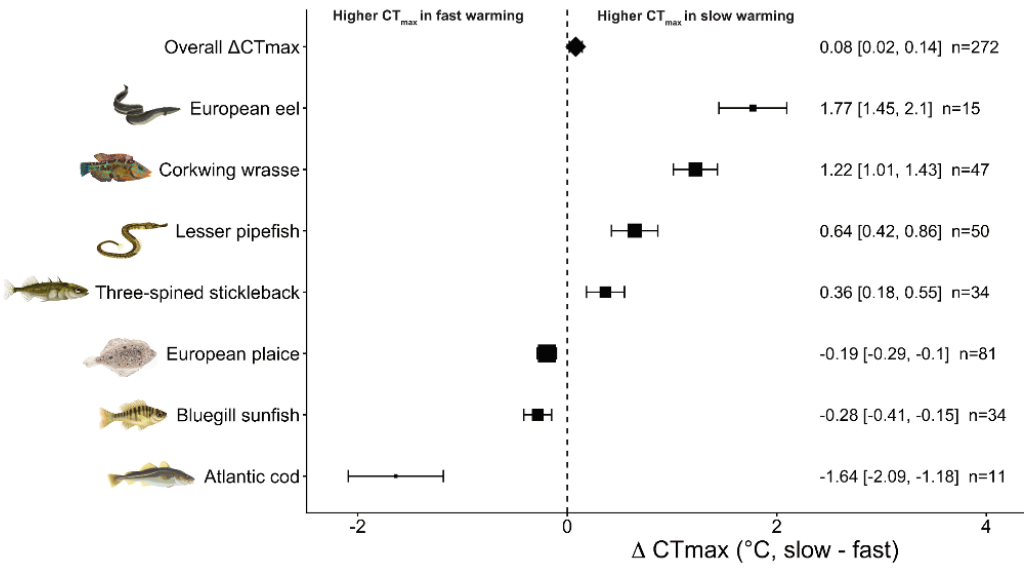

Figure 2. Species-specific differences in upper thermal tolerance (ΔCT_{max}, °C). Points show mean differences in CT_{max} between slow and fast warming rates for each species, with horizontal error bars representing 95% confidence intervals (CI). Positive values indicate higher CT_{max} under slow warming conditions relative to fast warming conditions. Point size is proportional to sample size (number of individuals, n). A dashed vertical line at zero indicates no difference between ramping rates. The diamond at the top represents the overall mean effect across species, with its width corresponding to the 95% confidence interval, weighted based on the variation in sampling variance. Numerical values of mean differences were displayed alongside each estimate for reference.

At the species level, fast CT_{max} was a strong predictor of slow CT_{max} across all seven species (between-species slope = 1.157; R^2 marginal = 0.873, R^2 conditional = 0.984; Figure 4; Figure 5). The between species slope of 1.157 being higher than 1, indicated that species with higher fast CT_{max} tended to show proportionally larger absolute differences between the two warming rates. Atlantic cod had both the lowest fast CT_{max} (24.53 ± 0.36°C, 95% CI) and slow CT_{max} (22.84 ± 0.30°C), while Bluegill sunfish had the highest fast CT_{max} (36.26 ± 0.13°C) and European eel the highest slow CT_{max} (36.22 ± 0.31°C). In contrast, the within-species slope was only 0.190, indicating that an individual's fast CT_{max} is a poor predictor of its slow CT_{max} within species (consistent with the weak within-species Spearman correlations described above).

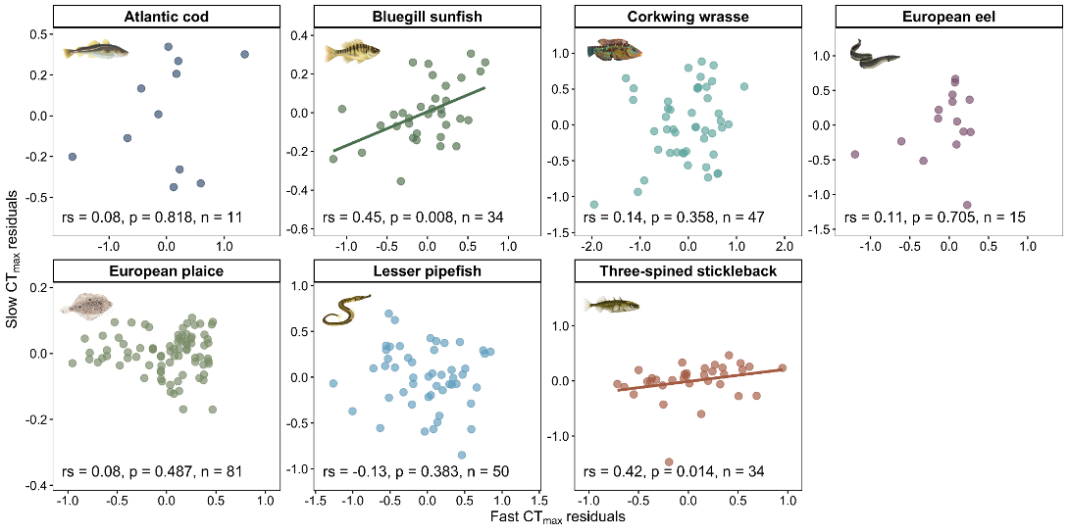

Figure 3. Spearman's Rank Correlation between fast and slow CT_{max} (°C) residuals of the seven study species. Correlation of model residuals corrects for species-specific fixed effects and differences among trial replicates (random effect) on CT_{max}. Inset values in each panel are the species' residual Spearman's Rank Correlation coefficient (r), the respective p-value (*p*), and sample size (*n*). Lines are linear regressions of species where a significant correlation was detected ($r > 0.2$, $p < 0.05$; bluegill sunfish and three-spined stickleback). Note the differing axis ranges among study species.

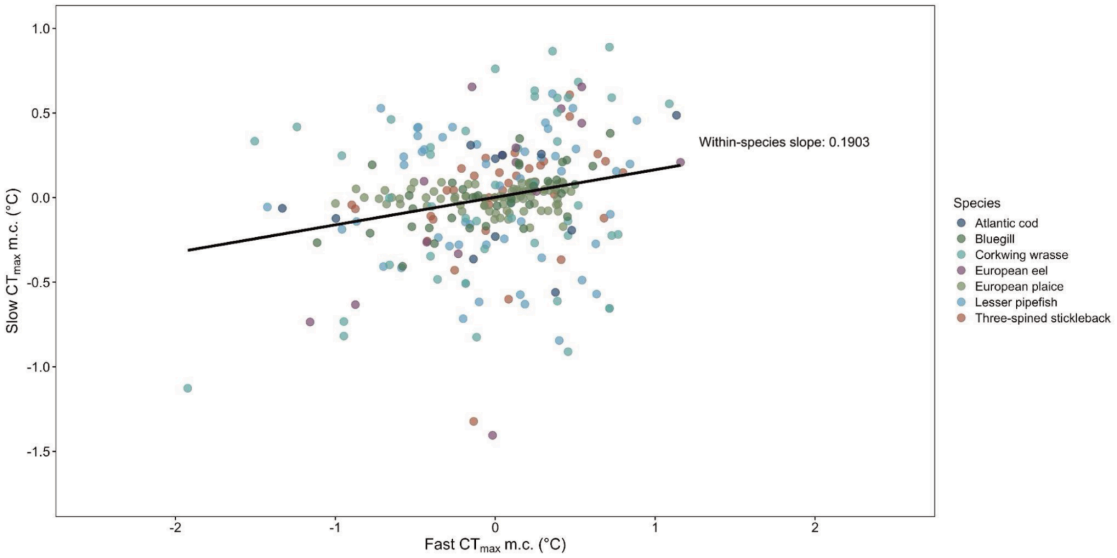

Figure 4. Study-wide Spearman's Rank Correlation between fast (0.3 min^{-1}) and slow (1°C h^{-1}) CT_{max} (°C). All seven study species grouped. A weakly positive correlation between fast and slow was detected (Spearman's rank: $R = 0.22$, $P < 0.001$, $n = 260$). CT_{max} values are corrected for trial effects using mean centering (m.c.), centering all values on their respective tank means but retaining raw variation. The bold line depicts the linear regression between fast and slow CT_{max} across the study.

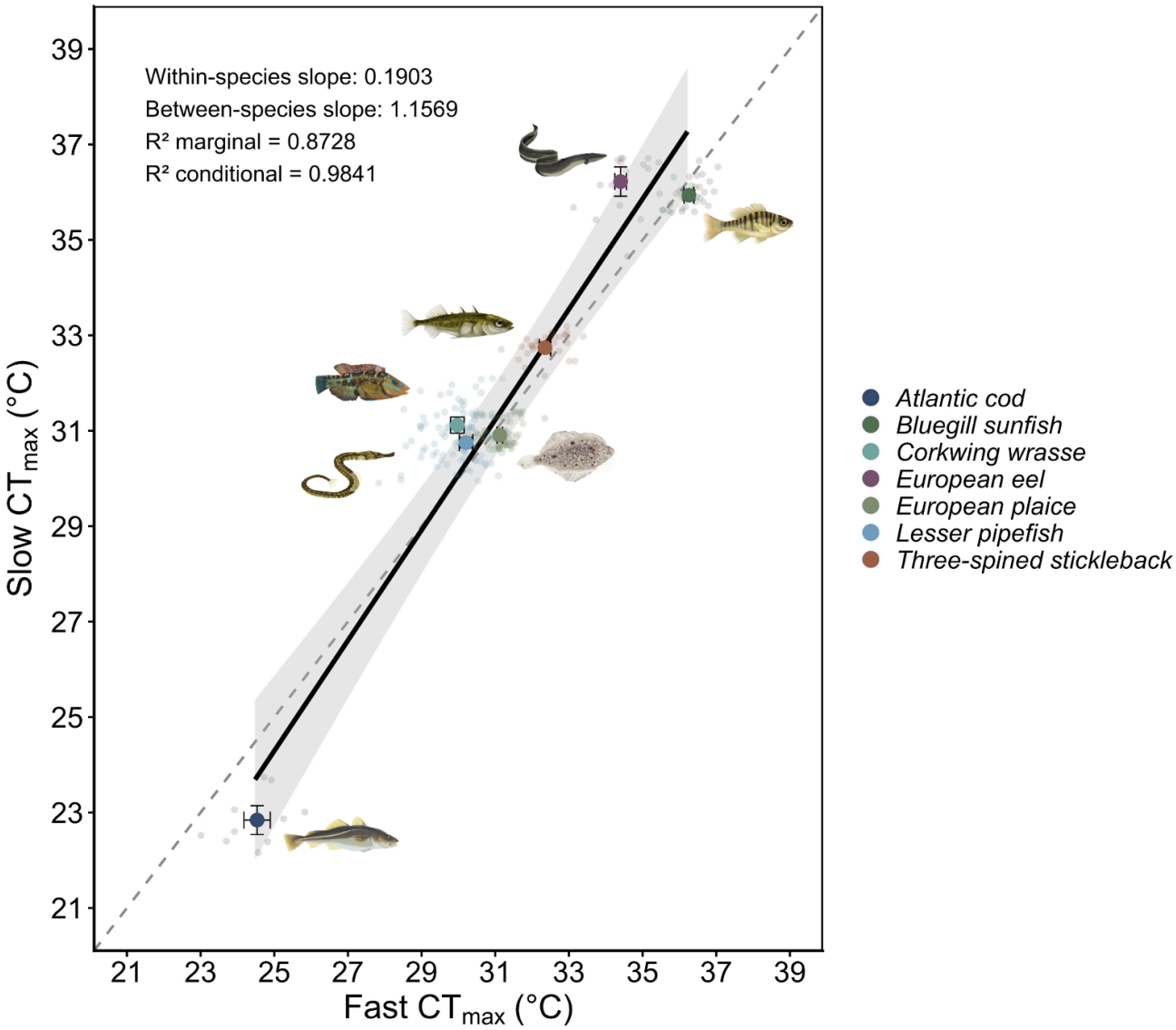

Figure 5. Species-level relationship between fast and slow CT_{max}. Transparent points show individual paired CT_{max} values (n = 272, paired across fast and slow trials via individual ID), coloured by species. Filled circles represent species means with 95% confidence intervals (± 1.96 SE) in both axes. The solid black line shows the population-level between-species slope from a linear mixed model (LMM; between-species slope = 1.157), with the shaded ribbon indicating the 95% CI of that prediction. The dashed grey line indicates the 1:1 line of equality. All species were acclimated to 16°C except Atlantic cod and bluegill sunfish who were acclimated to 9°C and 21°C, respectively.

Discussion

Co-variation between measures of thermal tolerance

Individual thermal tolerance measured under fast warming rates did not consistently predict thermal tolerance under slower warming rates across most species examined (Figure 1–5). Therefore, caution is needed when extrapolating individual CT_{max} temperatures to predict tolerance to more ecologically relevant rates of warming. Although significant positive correlations were detected in bluegill sunfish

and three-spined stickleback, these relationships were relatively weak, with fast-warming CT_{max} explaining only around 20% of the variation in slow-warming CT_{max}. Thus, even in species where fast- and slow-warming CT_{max} are significantly correlated, fast-warming CT_{max} provides only limited predictive power as a proxy for individual tolerance to slower warming. This low degree of concordance between ramping rates within individuals is somewhat surprising, as the mechanisms behind thermal tolerance are often assumed to be consistent across rates of warming (Ern et al., 2023) and there is repeatability in fast CT_{max} (Morgan et al., 2018). The discrepancy may indicate that the mechanisms differ depending on ramping rates, and individuals therefore show specific tolerances. It may also indicate that fast CT_{max} measurements are somewhat random in nature due to the short exposure times.

Contrary to the individual correlations, the between-species relationship between fast and slow CT_{max} was very strong, demonstrating that species with high fast-warming CT_{max} also tended to exhibit high slow-warming CT_{max} (Figure 5). This pattern can be driven by both evolutionary fixed species differences and differences in the acclimation temperatures. When considering only species acclimated to the same temperature (16°C), much of the relationship is driven by European eel and three-spined stickleback, suggesting that the overall between-species correlation may not be broadly representative, and is largely driven by the acclimation temperature. Regardless of the cause, the strength of the correlation means fast CT_{max} is highly usable for modelling and macroecological questions, with the caveat that it often overestimates tolerance to more sustained exposure to warming, as discussed below.

Effects of warming rate on CT_{max}

Our findings support concerns that fast-warming CT_{max} is unlikely to fully reflect physiological limits relevant to the warming that can occur in nature (Desforges et al., 2023; Ørsted et al., 2022). Fast CT_{max} trials are likely too brief for substantial thermal acclimation to occur (Ern et al., 2023; Metz et al., 2025) (Åsheim et al., 2020). However, the cellular heat shock response – which is part of a fish's thermal acclimation – can be initiated within minutes (Andreassen et al., 2025). Conversely, slower warming may allow for thermal acclimation, while also increasing cumulative heat injury (Ern et al., 2023).

In this study, the effect of warming rate varied widely among species (Figure 1). A handful of prior studies have been designed to focus on the effect of warming rate on thermal tolerance. Faster warming rates increased CT_{max} in Atlantic herring (*Clupea harengus*) but not in European seabass (*Dicentrarchus labrax*) (Moyano et al., 2017). Specifically, when exposed to warming rates ≤ 2°C h^{-1}, the CT_{max} of Atlantic herring larvae were on average ~4°C lower (with a range of 13°C) than those exposed to faster rates (4 and 8°C h^{-1}). In contrast, juvenile white sturgeon reached progressively higher CT_{max} values as the warming rate decreased, exhibiting a maximum tolerance at a very slow rate of 0.003°C min^{-1} (Penman et al., 2023). Additionally, in juvenile coho salmon (*Oncorhynchus kisutch*) and pumpkinseed sunfish (*Lepomis gibbosus*) this trend continued, where slower warming rates produced higher CT_{max} values (Becker & Genoway, 1979). However, in Atlantic salmon parr (*Salmo salar*) warming rates (0.02°C min^{-1} to 0.3°C min^{-1}) produced no difference in CT_{max} (Elliot & Elliott, 1995).

Therefore, our study aligns with previous work suggesting that estimates of slow- and fast-warming tolerance vary among species.

Variation in the effect of warming rate on thermal tolerance can be further contextualized by considering whether this variation is a function of the distance between the acclimation temperature and the critical temperature (T_{crit}; the highest sustainable acclimation temperature) for the animal. Variation in ΔCT_{max} among species could thus be predicted from where each species was acclimated relative to its thermal performance curve (TPC). Fish acclimated to low temperatures relative to their T_{crit} inherently have more capacity for mid-trial up-acclimation (i.e., physiological "room" available for mid-trial acclimation) of their warming tolerance than a fish already living near the upper (right) end of its TPC. Under this framework, species acclimated to a temperature further from their upper thermal limits might be expected to benefit more from the extended duration of slow warming trials. In slow trials, there could be a greater scope for acclimation responses to manifest before LOE is reached (Åsheim et al., 2020; Morgan et al., 2020; Ern et al., 2023), and, as acclimation temperature increases thermal tolerance plasticity decreases due to the shift closer to the T_{crit} (this shift and acclimation plateau representing concrete ceiling). When comparing current acclimation capacity data of Swedish marine species to our study's acclimation temperatures, the trend generally matches expectations. Wrasse and pipefish sit low on their TPCs and gain from slow warming; flatfish and three-spined stickleback sit near their upper limit and perform poorly under slow warming, as predicted. European eel performed best under slow warming with room for further acclimation (https://www.aquamaps.org/ summary in Tamarit-Castro et al., 2026). Atlantic cod and bluegill sunfish, however, had room for further acclimation but did not benefit from slow warming. Overall, most species' responses matched their predicted TPC position, though cod and bluegill deviated, suggesting factors beyond TPC position also shape warming-rate responses.

Differences in thermal tolerance among species

The fact that four of our species reached higher CT_{max} in slow warming trials as opposed to fast-warming trials indicates that the thermal death time (TDT) model is not universally applicable. In TDT models, the probability of death is a function of the duration and intensity of heat exposure, meaning that the cumulative "heat load" should inevitably result in lower tolerated temperatures during longer trials (Arnold et al., 2025; Bayat et al., 2025; Ørsted et al., 2022; Rezende et al., 2014). The reason it fails is likely because it does not account for within-trial acclimation, which increases with decreasing rates of warming (Ørsted et al., 2022; Ern et al., 2023). While TDT models can be relevant for modeling climate scenarios where heat stress accumulates over different time periods, our results suggest that their utility may be limited in teleosts capable of rapid acclimation, particularly when individuals are not already warm-acclimated. In these cases, the evidence presented here suggests acclimation during slow warming can more than offset the accumulation of thermal injury, resulting in higher, rather than lower, CT_{max}.

Importantly, our findings do not diminish the utility of fast CT_{max} assays. Rather, they demonstrate that CT_{max} estimates obtained under different warming rates are suited to different questions. Fast CT_{max} assays are valuable for quantifying acclimation dynamics and investigating physiological mechanisms

(Desforges et al., 2023; Ern et al., 2023; Raby et al., 2025a). Because fast- and slow-warming CT_{max} are strongly correlated across species (Figure 5), comparative studies aimed at ranking species by relative thermal tolerance are unlikely to be strongly affected by warming rate. However, fast CT_{max} should be used cautiously as a proxy for tolerance to slower, ecologically relevant warming, particularly when the objective is to predict individual performance, responses to environmental change, or to map future suitable habitat. In the latter case, especially (e.g., species distribution modeling), a difference of 0.5–1°C could be meaningful. However, ultimately, even slow-warming experiments remain simplified representations of natural thermal regimes, which are shaped by complex interactions among environmental variability, habitat use, food availability, behaviour, and thermal history (Iacarella et al., 2025).

Limitations

We chose to expose individuals to a fast CT_{max} protocol followed by a slow CT_{max} protocol, rather than a fully counterbalanced design. Previous work indicated that slow warming protocols are associated with high mortality (Desforges et al., 2023; Åsheim et al., 2020), which would have provided a challenge for both animal care and logistics of the experiment. The prolonged exposure to heat of having a slow-warming trial occur first would also add a layer of warm acclimation to the experiment that could carry-over to the second warming trial. However, the fixed order of exposure still meant potential for carryover effects from the initial fast CT_{max} trial—such as accumulated physiological stress, sublethal injury, or incomplete recovery. These carryover effects may have influenced tolerance estimates during the subsequent slow-warming exposure. Conversely, prior heat exposure may also have induced rapid thermal acclimation (i.e., heat hardening, an improvement in thermal tolerance following a previous heat exposure), potentially increasing CT_{max} during the subsequent slow-warming trial (Morgan et al., 2018; Metz et al., 2025). For example, an initial CT_{max} trial increased CT_{max} measured one week later by 0.2°C in Trinidadian guppies (*Poecilia reticulata*) (Grinder et al., 2020) and by 0.3°C in zebrafish (*Danio rerio*) (Morgan et al., 2018). Similarly, brook trout (*Salvelinus fontinalis*) exhibited a 0.16°C increase in CT_{max} one month after an initial CT_{max} trial (Stewart et al., 2023). If similar responses occurred in our study, they may have elevated the slow CT_{max} by fractions of a °C, but could not account for the large differences in CT_{max} found between ramping rates.

Conclusions

While CT_{max} is valuable, its relevance outside the context of its measurement (e.g., warming rate, acclimation temperature, species) varies. Individuals tolerant of fast warming are not necessarily also tolerant of slow warming; and the upper thermal limit at a slow warming rate is not always higher than the upper thermal limit at a fast-warming rate. Importantly, at the within-species level, the ecological relevance of CT_{max}-based inferences is context dependent and cannot be captured by a single warming rate. Instead, the relationship between tolerance to different rates of warming likely depends on the acclimation rate and capacity of a given species, the rate at which they accumulate heat injury and repair damage, and how consistent responses are among individuals—all of which should be explicitly considered in experimental design and applied modelling frameworks. The divergence between species-level and individual-level correlations between fast and slow CT_{max} further highlights that strong cross-species correlations can mask weak within-species relationships, and that the utility of fast

CT_{max} as a proxy for ecologically relevant thermal tolerance must be evaluated at the appropriate biological scale.

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Conflict of interest
The authors declare no competing or financial interests.

Author contributions
Lechner, E.R. and Stewart, E.M.C. designed the methodology, performed the formal analyses, and led the writing of the original manuscript draft. **Lechner, E.R., Stewart, E.M.C. and Pottier, P.** curated and visualized the data. **All authors** contributed to data collection and/or investigation. **Binning, S.A., Jutfelt, F.W., Lechner, E.R., Stewart, E.M.C. and Cowan, Z.-L.** conceived the study, contributed to the conceptualization and study design. **Binning, S.A., Jutfelt, F.W. and Raby, G.D.** provided supervision. **All authors** contributed critically to revising and editing the manuscript. All authors gave final approval for publication.

Data availability
The data (10.6084/m9.figshare.33060812) and R scripts (10.6084/m9.figshare.33060797) used for the analysis in this study, along with data documentation (10.6084/m9.figshare.33060770) and reference videos (10.6084/m9.figshare.33060758), are openly accessible on FigShare. These materials were shared with the reviewers and editors on manuscript submission.

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Supplementary Material

Supplementary Results

1. *Body mass*

There were associations between body mass and CT_{max} in three of the seven species: bluegill, lesser pipefish, and three-spined stickleback. CT_{max} increased subtly with increasing body mass in bluegill in both fast and slow trials (fast $\beta_{mass} = 0.060 \pm 0.025$, $p = 0.024$; slow $\beta_{mass} = 0.041 \pm 0.011$, $p = 0.001$; Figure S4). In lesser pipefish and three-spined stickleback, we found a negative effectbody mass on CT_{max} , but only at one warming rate. Three-spined stickleback CT_{max} decreased by 0.788°C for every 1 g increase in body mass at a fast warming rate (fast $\beta_{mass} = -0.788 \pm 0.300$, $p = 0.011$, Figure S4), but not under a slow warming rate (slow $\beta_{mass} = 0.063 \pm 0.248$, $p = 0.801$, Figure S4). Conversely, in lesser pipefish, CT_{max} decreased with increasing body mass under slow warming (slow $\beta_{mass} = -0.534 \pm 0.200$, $p = 0.010$, Figure S4), and there was a similar trend at the fast warming rate, albeit not statistically significant (fast $\beta_{mass} = -0.429 \pm 0.273$, $p = 0.122$, Figure S4). Notably, in European eel, we found a positive association between body mass and slow CT_{max} that approached significance (slow $\beta_{mass} = 5.862 \pm 2.731$, $p = 0.057$, Figure S4), but no similar relationship for fast CT_{max} (fast $\beta_{mass} = -1.000 \pm 1.466$, $p = 0.500$, Figure S4).

2. *Endpoint type*

Multiple endpoints were identified and recorded in European eel and three-spined stickleback. European eel marked as demonstrating one of the two alternative endpoints (non responsive, NR; or not breathing, NB) in fast trials had 0.833°C and 0.958°C higher CT_{max} (fast $\beta_{NR} = 0.833 \pm 0.260$, $p = 0.003$; fast $\beta_{NB} = 0.958 \pm 0.312$, $p = 0.004$, Table S2; Figure S5A). For these same individuals in slow trials, the 'not breathing' endpoint was not observed, and those marked as non responsive did not have

a significantly different CT_{max} (slow $\beta_{NR} = 0.262 \pm 0.308$, $p = 0.413$). An alternative endpoint (non responsive, NR) was only noted in three-spined stickleback in fast trials, but those marked as non responsive did not differ in CT_{max} (fast $\beta_{NR} = 0.065 \pm 0.162$, $p = 0.689$, Table S2; Figure S5B).

3. Lesser pipefish gravidity

There were 25 lesser pipefish individuals noted as gravid during CT_{max} trials (24 measured in both warming rates). Gravidity was not significantly associated with CT_{max} at either warming rate (fast $\beta_{gravid} = 0.149 \pm 0.159$, $p = 0.354$; slow $\beta_{gravid} = -0.090 \pm 0.107$, $p = 0.403$, Table S2; Figure S5C).

4. Lethality

Individuals that did not recover within two hours following a CT_{max} trial were marked as mortalities. Mortalities occurred following six of the fourteen sets of trials (European eel slow, European plaice fast and slow, corkwing wrasse slow, lesser pipefish fast and slow). We found significant variation in CT_{max} among mortality groups in two of these cases, both in slow warming trials, although the overall outcome was not changed. Corkwing wrasse that did not recover from the slow trial had a 0.843°C higher CT_{max} ($n = 3$, Table S2; Figure S5D). The effect was more subtle in European plaice—individuals that did not recover from the slow trial had only a 0.071°C higher CT_{max} than those that recovered ($n = 5$; Table S2; Figure S5F).

Table S1. Collection methods, holding conditions, feeding regimes and photoperiods for all study species. S.D. refers to standard deviation.

Species	Collection Method	Collection Dates (2025)	Holding Conditions	Acclimation Temp (°C) & Duration	Feeding (pre-CTmax)	Photoperiod (light/dark)	Mass and length ± S.D.
Atlantic cod (*Gadus morhua*)	Baited cages in harbour area	April 26–May 28	420–1350 L tanks, flow-through seawater	9°C, 10 days pre-study	Shrimp, cod, pollock	16/8 h (artificial)	180.47 ± 132.78 g 258 ± 59.78 mm
Bluegill sunfish (*Lepomis macrochirus*)	Beach seine in shallow vegetated areas	Sept 8–11	500 L tanks, sand-filtered freshwater, biofilter & UV	~21°C, 14–19 days pre-study	Blood worms, mysid shrimp	12/12 h (later 9/15 h)	11.01 ± 2.6 g
Corkwing wrasse (*Symphodus melops*)	Beach seine in shallow vegetated areas, baited cages in harbour area	June 12–17	19–25 L tanks, flow-through seawater	~16°C, 4–20 days	Raw prawn	18/6 h	8.83 ± 2.95 g 93 ± 9.32 mm
European eel (*Anguilla anguilla*)	Elver traps	June 16	1–1.5 L tanks, flow-through seawater	~16°C, 4–20 days	Artemia, mysis shrimps, red mosquito larvae (twice daily)	20/4 h	0.25 ± 0.05 g 72.73 ± 3.24 mm
European plaice (*Pleuronectes platessa*)	Beach seine in shallow sandy areas	June 11–17	5 L tanks, flow-through seawater	~16°C, 4–20 days	Artemia	18/6 h	0.53 ± 0.17 g 37.36 ± 4.17 mm
Lesser pipefish (*Syngnathus rostellatus*)	Beach seine in shallow vegetated areas	June 12–17	4.6 L tanks, flow-through seawater	~16°C, 4–20 days	Artemia	18/6 h	0.68 ± 0.26 g 121.56 ± 17.07 mm

Three-spined stickleback (*Gasterosteus aculeatus*)	Beach seine in shallow vegetated areas	June 18–22	~5 L tanks, flow-through seawater	~16°C, 4–20 days	Artemia, raw prawn	18/6 h	1.26 ± 0.25 g 53.58 ± 3.69 mm

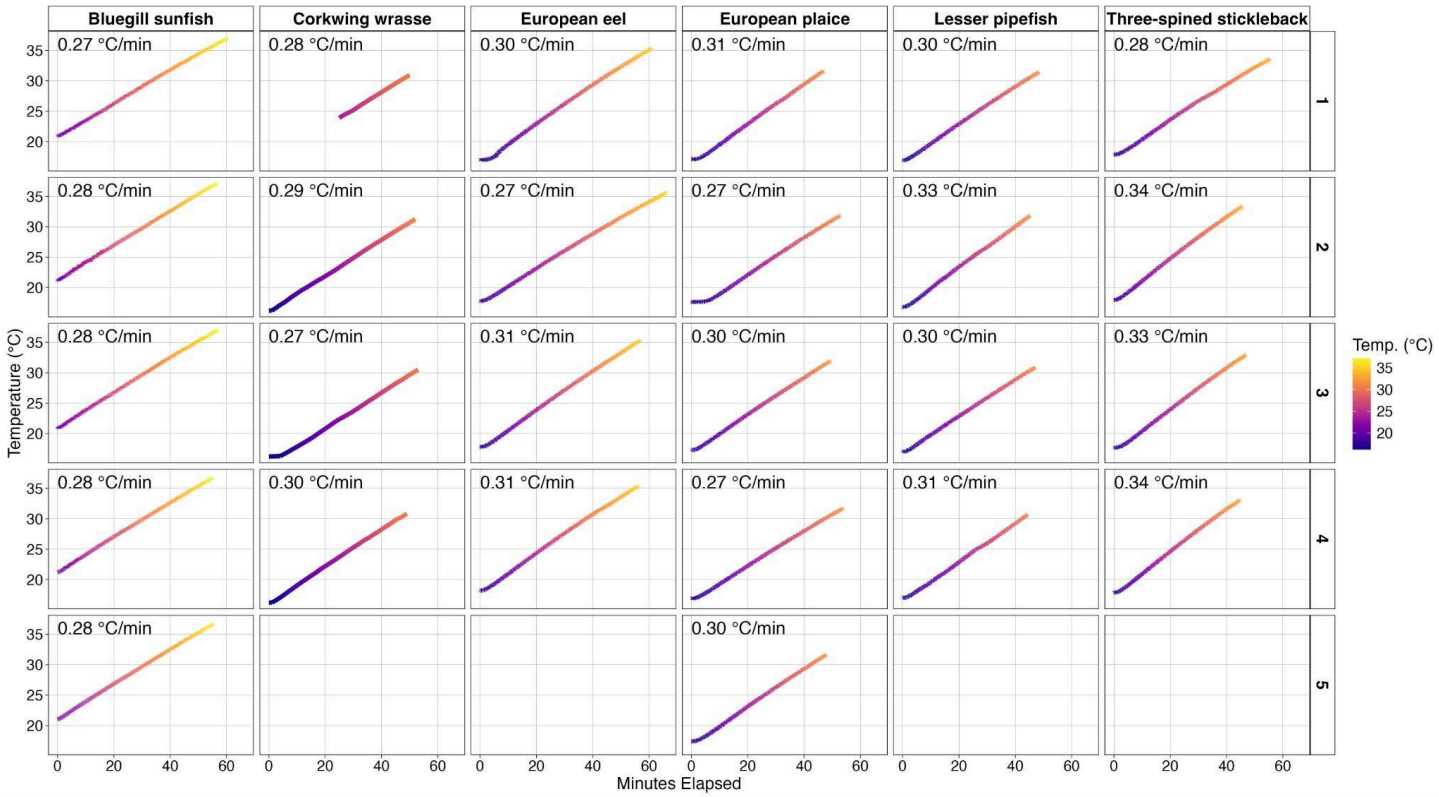

Figure S1. Logged fast CT_{max} trial warming rates (18°C/hour, 0.3°C/min). Species and trial number (1–5) identified by the top and right side labels, respectively. Trial temperatures were recorded using RBR Solo^3 loggers or HOBO pendants (see *Methods*, Metadata on FigShare). The warming rate in the top left of each panel reflects the calculated warming rate between the trial start and the final CT_{max} of the trial. Data in corkwing wrasse trial 1 is trimmed as the logger was added to the arena mid-trial. See Figure S3 for Atlantic cod warming rates.

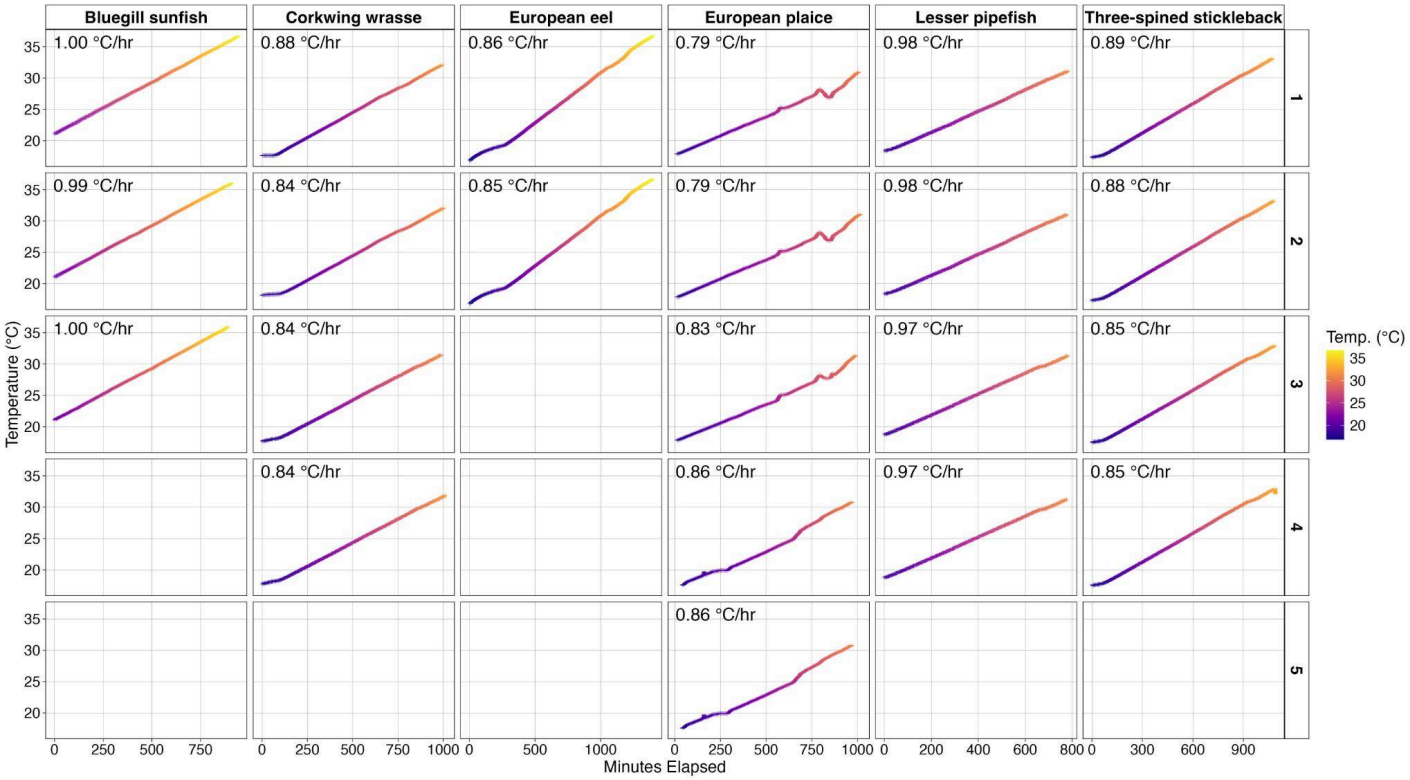

Figure S2. Logged slow CT_{max} trial warming rates (1°C/hour). Species and trial number (1–5) identified by the top and right side labels, respectively. Trial temperatures were recorded using RBR Solo^3 loggers or HOBO pendants (see *Methods*, Metadata on FigShare). The warming rate in the top left of each panel reflects the calculated warming rate between the trial start and the final CT_{max} of the trial. Data in European plaice trials are trimmed as the loggers were added to the arena 10–30 minutes after the trials commenced. European plaice trials 1, 2, and 3 were the first attempt at using the automatic warming software, thus adjustments in software settings occurred around 800 minutes, causing the slight variation in rate. An equipment malfunction caused the warming rates to rapidly increase at the end of European eel trials 3 and 4, therefore, these trials are not included in analyses. See Figure S3 for Atlantic cod warming rates.

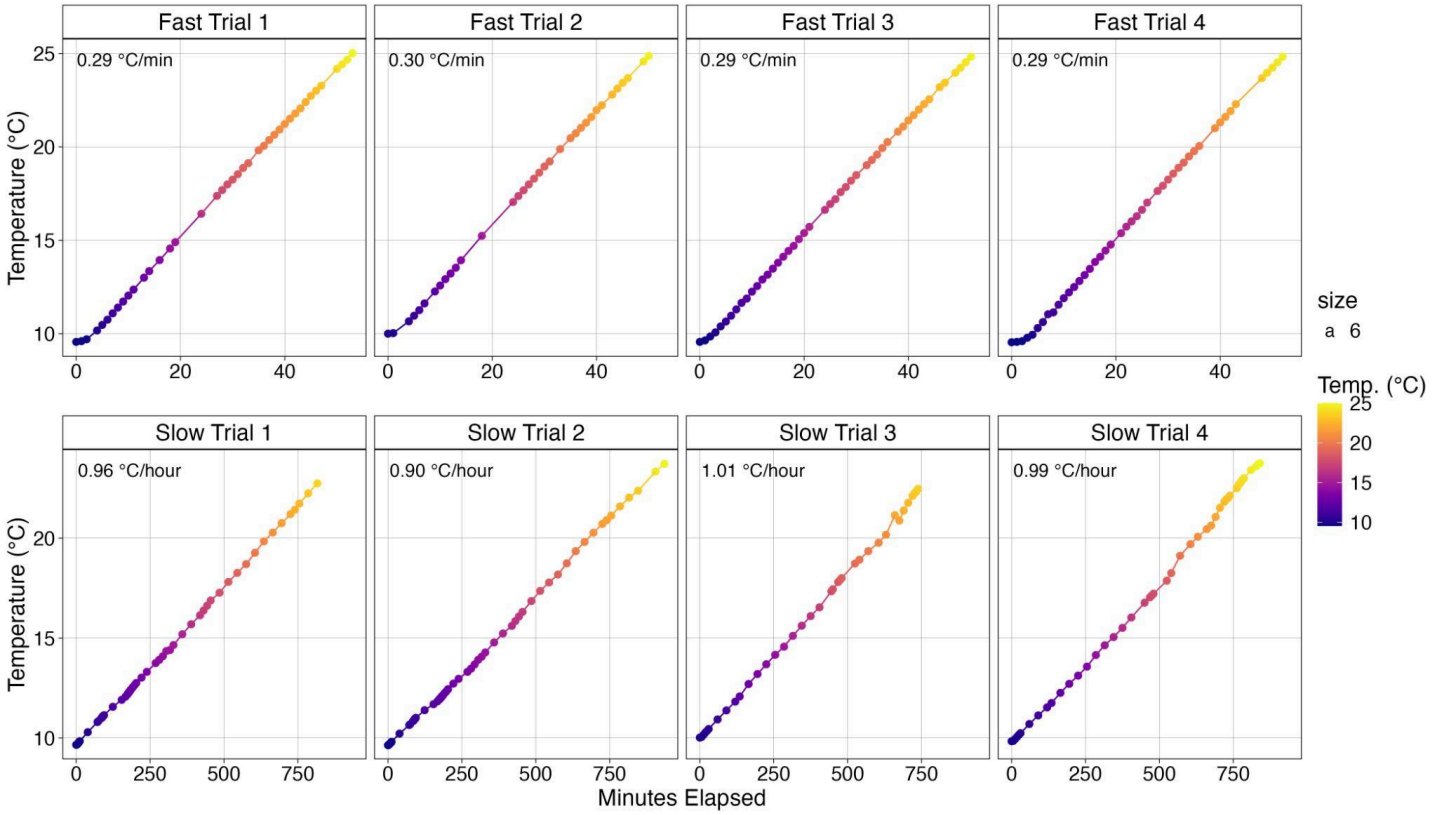

Figure S3. Manually recorded warming rates for Atlantic cod CT_{max} trials. Individual trials are labelled by rate (fast or slow) and trial number (1–4). Temperature throughout the trial was continuously monitored using a fiber-optic oxygen sensor and temperature sensor (FireSting-PRO, Pyro Science); plotted points represent time points at which the temperature was manually logged from these measurements. The warming rate in the top left of each panel reflects the calculated warming rate between the trial start and the final CT_{max} of the trial.

Table S2. Linear mixed effect model coefficients for **A)** Atlantic cod, **B)** bluegill sunfish, **C)** corkwing wrasse, **D)** European eel, **E)** European plaice, **F)** lesser pipefish, and **G)** three-spined stickleback fast and slow CT_{max}. Fast and slow CT_{max} were modelled independently for each species and with any species-specific relevant fixed effects (see *Methods*). Fixed effects included mass (g), mortality (factor with 2 levels: non-lethal, lethal), endpoint type (factor with 2 or 3 levels: LOE, non responsive, not breathing), and gravidity (factor with 2 levels: not gravid, gravid). Trial 'replicate' was included as a random effect in all models. For each fast and slow model, the tested effects (term), effect coefficients (β ± standard error, SE), 95% confidence intervals (intercept and fixed effects only; 95% CI), p-value (intercept and fixed effects only; p, with significance notation), adjusted and conditional interclass correlation coefficients (random effect of trial replicate only; Trial SD; ICC adj. and ICC cond., respectively), and marginal and conditional R^2 (R^2 marg. and R^2 Cond., respectively) are reported.

Species	Rate	Term	β ± SE	95% CI	p		ICC Adj.	ICC Cond.	R^2 Marg.	R^2 Cond.
A) Atlantic cod	**Fast**	(Intercept)	24.767 ± 0.333	(23.582, 25.952)	0	***	-	-	-	-

		Mass	-0.001 ± 0.001	(-0.005, 0.002)	0.414	.	-	-	-	-
		Trial SD	0.132	-	-	.	0.034	0.033	-	-
		σ	0.7	-	-	.	-	-	-	-
									0.055	0.088
	Slow	(Intercept)	23.133 ± 0.351	(22.204, 24.061)	0	***	-	-	-	-
		Mass	-0.001 ± 0.001	(-0.004, 0.002)	0.331	.	-	-	-	-
		Trial SD	0.359	-	-	.	0.466	0.412	-	-
		σ	0.385	-	-	.	-	-	-	-
									0.114	0.527
B) Bluegill sunfish	**Fast**	(Intercept)	35.599 ± 0.287	(35.016, 36.182)	0	***	-	-	-	-
		Mass	0.06 ± 0.025	(0.008, 0.111)	0.024	*	-	-	-	-
		Trial SD	0.058	-	-	.	0.019	0.017	-	-
		σ	0.422	-	-	.	-	-	-	-
									0.117	0.133
	Slow	(Intercept)	35.467 ± 0.202	(34.927, 36.007)	0	***	-	-	-	-
		Mass	0.041 ± 0.011	(0.019, 0.063)	0.001	***	-	-	-	-
		Trial SD	0.281	-	-	.	0.746	0.666	-	-
		σ	0.164	-	-	.	-	-	-	-
									0.108	0.774
C) Corkwing wrasse	**Fast**	(Intercept)	29.622 ± 0.343	(28.879, 30.365)	0	***	-	-	-	-
		Mass	0.039 ± 0.028	(-0.018, 0.096)	0.173	.	-	-	-	-
		Trial SD	0.438	-	-	.	0.345	0.337	-	-
		σ	0.603	-	-	.	-	-	-	-
									0.024	0.361
	Slow	(Intercept)	30.883 ± 0.27	(30.321, 31.444)	0	***	-	-	-	-
		Mass	0.022 ± 0.026	(-0.032, 0.075)	0.417	.	-	-	-	-
		Lethal	0.843 ± 0.32	(0.197, 1.488)	0.012	*	-	-	-	-

		Trial SD	0.198	-	-	.	0.126	0.11	-	-
		σ	0.521	-	-	.	-	-	-	-
									0.128	0.238
D) European eel	**Fast**	(Intercept)	34.538 ± 0.365	(33.798, 35.278)	0	***	-	-	-	-
		Mass	-1 ± 1.466	(-3.973, 1.973)	0.5	.	-	-	-	-
		Non responsive endpoint	0.833 ± 0.26	(0.306, 1.36)	0.003	**	-	-	-	-
		Not breathing endpoint	0.958 ± 0.312	(0.325, 1.591)	0.004	**	-	-	-	-
		Trial SD	0	-	-	.	-	-	-	-
		σ	0.429	-	-	.	-	-	-	-
									0.323	-
	Slow	(Intercept)	34.538 ± 0.751	(32.882, 36.195)	0	***	-	-	-	-
		Mass	5.862 ± 2.731	(-0.222, 11.947)	0.057	.	-	-	-	-
		Non responsive endpoint	0.262 ± 0.308	(-0.418, 0.942)	0.413	.	-	-	-	-
		Lethal	0.487 ± 0.442	(-0.498, 1.472)	0.297	.	-	-	-	-
		Trial SD	0.082	-	-	.	0.022	0.015	-	-
		σ	0.546	-	-	.	-	-	-	-
									0.297	0.312
E) European plaice	**Fast**	(Intercept)	31.122 ± 0.142	(30.832, 31.412)	0	***	-	-	-	-
		Mass	-0.012 ± 0.227	(-0.463, 0.439)	0.959	.	-	-	-	-
		Lethal	0.219 ± 0.218	(-0.213, 0.651)	0.318	.	-	-	-	-
		Trial SD	0.153	-	-	.	0.16	0.158	-	-
		σ	0.35	-	-	.	-	-	-	-
									0.01	0.168
	Slow	(Intercept)	30.902 ± 0.121	(30.576, 31.228)	0	***	-	-	-	-
		Mass	-0.039 ± 0.046	(-0.13, 0.053)	0.399	.	-	-	-	-
		Lethal	0.071 ± 0.031	(0.01, 0.133)	0.024	*	-	-	-	-

		Trial SD	0.265	-	-	.	0.945	0.94	-	-
		σ	0.064	-	-	.	-	-	-	-
									0.005	0.945
F) Lesser pipefish	**Fast**	(Intercept)	30.472 ± 0.347	(29.608, 31.336)	0	***	-	-	-	-
		Mass	-0.429 ± 0.273	(-0.976, 0.118)	0.122	.	-	-	-	-
		Lethal	0.182 ± 0.255	(-0.329, 0.693)	0.478	.	-	-	-	-
		Gravid	0.149 ± 0.159	(-0.17, 0.467)	0.354	.	-	-	-	-
		Trial SD	0.573	-	-	.	0.531	0.519	-	-
		σ	0.539	-	-	.	-	-	-	-
									0.022	0.542
	Slow	(Intercept)	31.161 ± 0.154	(30.846, 31.477)	0	***	-	-	-	-
		Mass	-0.534 ± 0.2	(-0.937, -0.132)	0.01	*	-	-	-	-
		Lethal	-0.369 ± 0.262	(-0.896, 0.158)	0.165	.	-	-	-	-
		Gravid	-0.09 ± 0.107	(-0.305, 0.125)	0.403	.	-	-	-	-
		Trial SD	0.121	-	-	.	0.108	0.086	-	-
		σ	0.349	-	-	.	-	-	-	-
									0.2	0.286
G) Three-spined stickleback	**Fast**	(Intercept)	33.32 ± 0.399	(32.518, 34.123)	0	***	-	-	-	-
		Mass	-0.788 ± 0.3	(-1.389, -0.187)	0.011	*	-	-	-	-
		Non responsive	0.065 ± 0.162	(-0.26, 0.39)	0.689	.	-	-	-	-
		Trial SD	0.178	-	-	.	0.099	0.088	-	-
		σ	0.537	-	-	.	-	-	-	-
									0.109	0.197
	Slow	(Intercept)	32.66 ± 0.321	(32.005, 33.314)	0	***	-	-	-	-
		Mass	0.063 ± 0.248	(-0.442, 0.568)	0.801	.	-	-	-	-
		Trial SD	0	-	-	.	-	-	-	-

	σ	0.348	-	-	.	-	-	-	-
								0.002	-

Table S3. Spearman's Rank Correlation between fast and slow CT_{max} (°C) residuals of the seven study species. Correlation of model residuals corrects for species-specific fixed effects and differences among trial replicates (random effect) on CT_{max}. Test values summarized include species' sample size (n), residual Spearman's Rank Correlation coefficient (r), S, p-value (p), and significance notation.

Species	**n**	**r**	**S**	**p**	
Atlantic cod	11	0.08	202	0.818	.
Bluegill sunfish	34	0.45	3600	0.008	**
Corkwing wrasse	47	0.14	14928	0.358	.
European eel	15	0.11	500	0.705	.
European plaice	81	0.08	81632	0.487	.
Lesser pipefish	50	-0.13	23442	0.383	.
Three-spined stickleback	34	0.42	3804	0.014	*

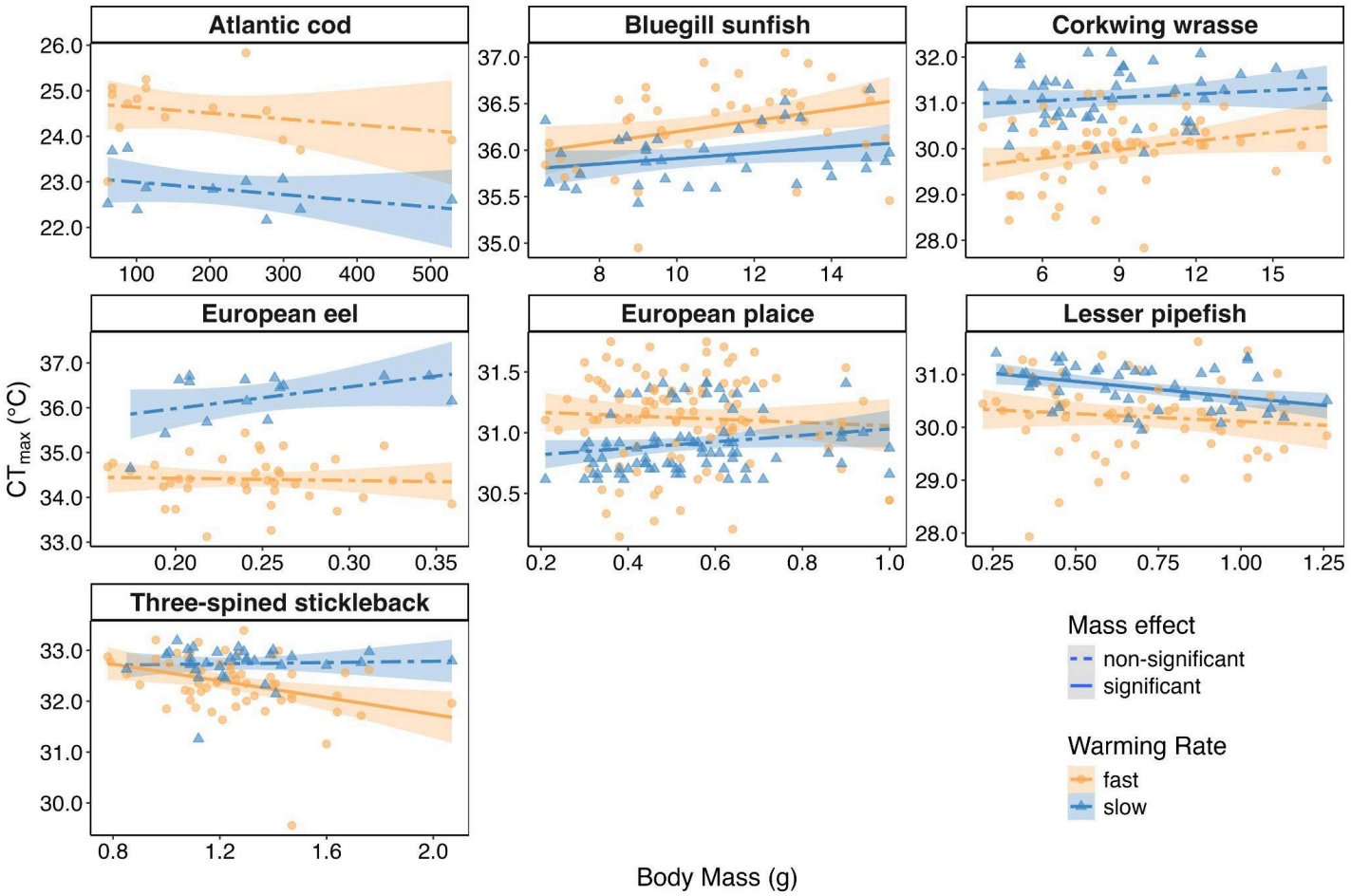

Figure S4. Effect of body mass (g) on CT_{max} (°C) during fast (orange circles, 0.3°C min^{-1}) and slow (blue triangles, 1°C h^{-1}) warming trials. Trend lines are linear regressions (*ggplot* method = "lm") with confidence intervals (shading). Lines are solid where a significant body mass effect on CT_{max} was detected, otherwise dashed. Points represent individual fish within each warming rate. Note the differing y-axis range among study species.

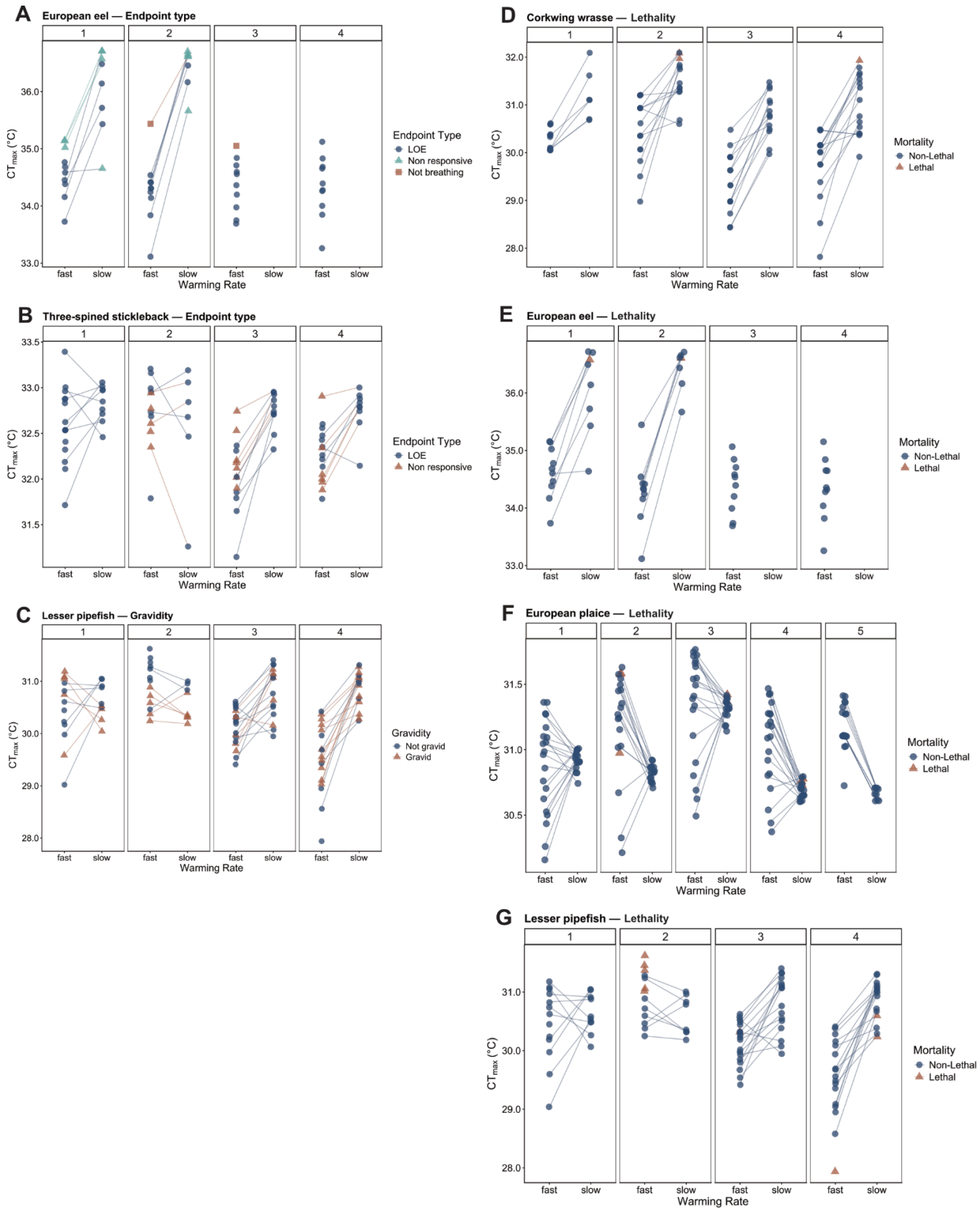
A
European eel — Endpoint type
1
2
3
4
36.0
35.0
34.0
33.0
CTmax (°C)
fast
slow
Warming Rate
Endpoint Type
LOE
Non responsive
Not breathing
B
Three-spined stickleback — Endpoint type
33.5
33.0
32.5
32.0
31.5
Endpoint Type
LOE
Non responsive
C
Lesser pipefish — Gravidity
31.0
30.0
29.0
28.0
Gravidity
Not gravid
Gravid
D
Corkwing wrasse — Lethality
32.0
31.0
30.0
29.0
28.0
Mortality
Non-Lethal
Lethal
E
European eel — Lethality
36.0
35.0
34.0
33.0
Mortality
Non-Lethal
Lethal
F
European plaice — Lethality
5
31.5
31.0
30.5
Mortality
Non-Lethal
Lethal
G
Lesser pipefish — Lethality
31.0
30.0
29.0
28.0
Mortality
Non-Lethal
Lethal

Figure S5. Fixed effects of endpoint type, gravidity and lethality on fast and slow CTmax (°C) across a subsection of study species. A-B: Shape indicates whether the endpoint was a traditional LOE (circle) or not (diamond or square). **C:** Shape indicates whether the fish was not gravid (circle) or gravid (triangle) based on visual inspection post-trial. **D-G:** Points indicate individual fish and shape indicates whether the trial was non-lethal (circle) or lethal (triangle). Slow trials 3 and 4 for European eel (E) were not included in analysis due to an equipment malfunction affecting the warming rate. Each point represents an individual fish, and lines join an individual's paired fast and slow warming CT_{max} values, if it recovered from both trials. Note the differing y-axis range does not start at 0.

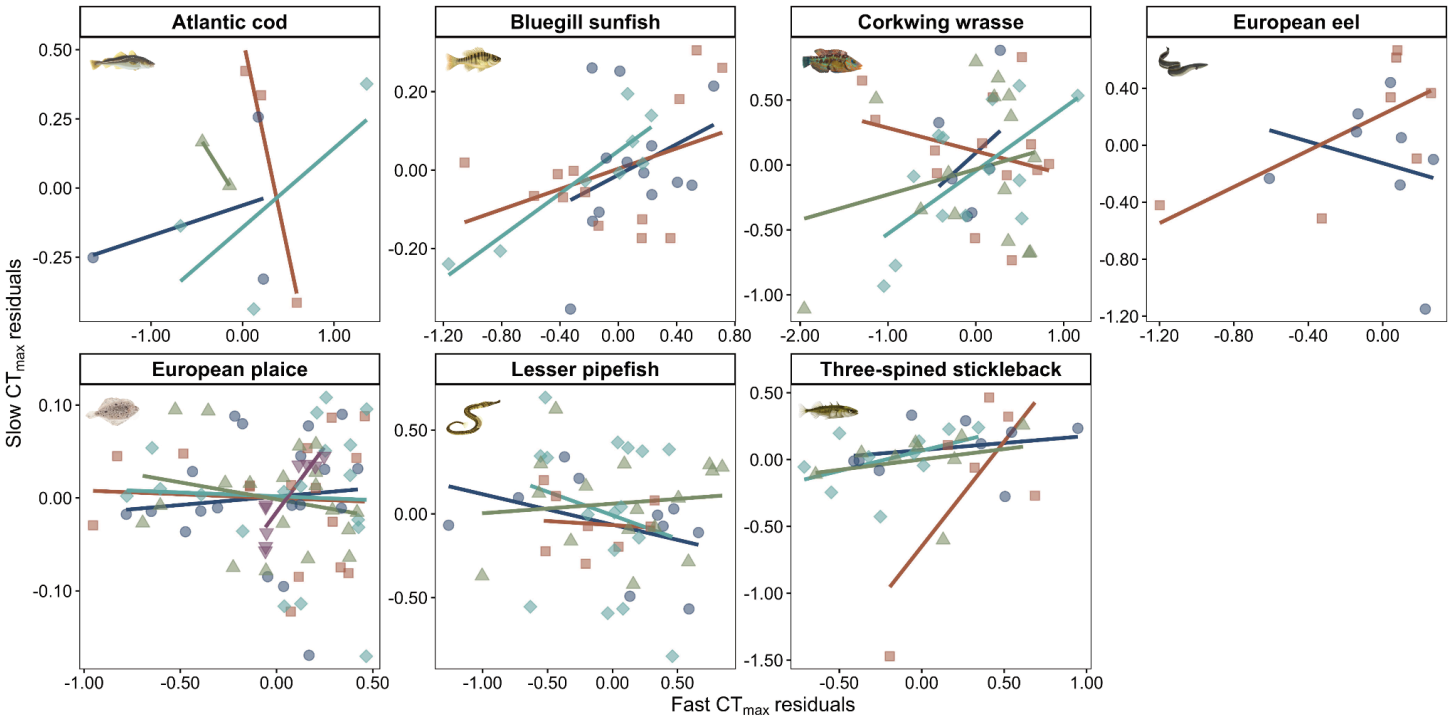

Figure S6. Fast vs. slow CT_{max} (°C) model residuals by trial replicate for the seven study species. A species-level correlation between the two traits was only detected in bluegill sunfish and three-spined stickleback). Points represent individual fish and trial replicates within species are distinguished by point colour/shape. Trend lines are linear regressions (*ggplot* method = "lm") to visualize trial replicate trends, but do not represent significant correlations between trials. Correlation of model residuals was not analyzed at the trial level. Note that the axes vary by species.

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