# Oxygen supersaturation has negligible effects on warming tolerance in aquatic ectotherms

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## Abstract

Under the midday sun when photosynthesizers are producing oxygen, shallow aquatic ecosystems can become supersaturated with oxygen (>100% air saturation) while they simultaneously peak in water temperature. It has been suggested that oxygen supersaturation could protect water-breathing animals from mortality during heatwaves because of the potential role of oxygen in governing thermal tolerance. Here, we conducted a circumglobal assessment of the effects of ecologically relevant oxygen supersaturation (150%, hyperoxia) on warming tolerance ($CT_{max}$) in 14 aquatic ectotherms from diverse marine and freshwater environments (ten fishes, four decapod crustaceans), in a series of 24 experiments that included 147 $CT_{max}$ trials and 1451 animals using two different warming rates (0.3°C $min^{-1}$ and 1°C $h^{-1}$). In 10 of 14 species, there was no effect of oxygen supersaturation relative to normoxic controls. In four species (two tropical reef fishes and two marine decapod crustaceans) we found mixed evidence for effects of oxygen saturation, with most of the effects being small (*ca*. 0.2–0.3°C). Thus, contrary to predictions, we conclude that oxygen supersaturation is unlikely to protect most water breathers from heatwaves and therefore few species distribution models or thermal risk assessments will benefit from incorporating oxygen supersaturation.

## Introduction

Shallow aquatic environments are among the most extreme and variable on the planet. The abiotic conditions in tidal pools, reef flats, saltmarshes, shallow lake habitats, and streams can change rapidly due to events such as tidal cycles, floods, and phytoplankton blooms. Moreover, climate change and the associated increase in heatwaves are amplifying acute heat stress in many of these aquatic ecosystems, threatening the performance and persistence of resident animals [1].

During daytime, when water temperatures are typically peaking and, in some cases, threatening aquatic animals via heat stress [2], many photosynthetic organisms also reach peak photosynthesis and oxygen production [3]. As a result, oxygen supersaturation (hyperoxia, i.e., dissolved oxygen partial pressures >100% air saturation) regularly occurs in shallow water bodies, commonly reaching levels around 150% of air saturation [3–5] (Supplementary Table S1). A leading hypothesis in climate change biology is that the warming tolerance of fish and other ectotherms is limited by oxygen transport capacity [2,6–8]. The "oxygen-limitation" hypothesis proposes that warming creates a mismatch between the temperature-induced rise in metabolic oxygen demand and the capacity of the cardiorespiratory system to supply tissues with oxygen, causing tissue hypoxia and ultimately loss of vital functions [2,6,7]. The simultaneous peaks in temperature and oxygen in shallow water environments give rise to the possibility that natural cycles in aquatic oxygen levels could help to protect water-breathing ectotherms by increasing oxygen supply and, in turn, enabling the maintenance of performance or survival during periods of high temperature [3].

Relatively few studies have tested for the effect of hyperoxia on warming tolerance in aquatic animals, but some data exist. For example, McArley et al. [9] reviewed experiments on fish and reported benefits of hyperoxia for warming tolerance in 9 of 20 species tested (also see [*10, 11*]). The mean improvement in critical thermal maximum ($CT_{max}$) across those studies was *ca*. 0.90°C (at 140–200% air saturation) relative to normoxic controls (i.e., ~100% air saturation) [9], bearing in mind that hyperoxia can become detrimental to fishes when oxygen levels approach 200% [12]. Notably, sample sizes were usually small at 8–10 animals per treatment [9] and typically with only n=1 replicate $CT_{max}$ trial. Some data on aquatic invertebrates have been reported as well. In nymphs of the mayfly *Seratella ignita* exposed to hyperoxia (~285% air saturation), a 1.2°C increase in $CT_{max}$ occurred relative to normoxia, but no significant difference was reported for the nymphs of *Ephemera danica* [13]. While inconsistent and small effects of hyperoxia on warming tolerance suggest a nuanced rather than universal benefit to aquatic animals, a study by Giomi et al. [3] stands out as reporting the largest and clearest effect sizes. During a 2°C $h^{-1}$ warming experiment, hyperoxia (140% air saturation) increased warming tolerance by an average of 2.25°C (range 1.2–3.5°C) across six marine species from the Red Sea (two fishes, four invertebrates, [3]). All six species live in tropical coastal habitats where oxygen supersaturation and rising sea temperature exhibit similar diurnal cycles, and thus the authors concluded that naturally occurring hyperoxia can protect aquatic animals during heatwaves [3]. Thus, conflicting results across a relatively limited body of evidence highlight the need for a large-scale empirical assessment of whether warming tolerance is limited by oxygen (and by how much), using consistent methods and a broad array of species.

Here, we assessed the universality of the potential benefit of naturally occurring oxygen supersaturation among marine and freshwater ectotherms via a multi-lab and multi-continental investigation. To do so, we assessed the effect of hyperoxia (150% air saturation) on the warming tolerance of 14 species of aquatic ectotherms. The 14 species included ten fishes and four decapod crustaceans from a variety of shallow temperate and tropical aquatic habitats (e.g., tide pools and the shallow areas of coral reefs, lakes, rivers, and streams) at varying latitudes, each of which are likely to exhibit oxygen supersaturation similar to the levels used here (Fig. 1; Supplementary Table S1 and Fig. S2). Warming tolerance was assessed using $CT_{max}$ trials (the temperature at which loss of motor function occurs during acute warming) at the recommended warming rate of 0.3°C $min^{-1}$ [14]. Furthermore, to encompass the rates of warming used in previous studies [3,9,11] and investigate if warming rates interact with an oxygen limitation, eight species were also tested using a slower warming rate of 1°C $h^{-1}$. By measuring the individual warming tolerance of 1451 animals (Supplementary Table S2) across 24 experiments and 147 CT$_{max}$ trials, the data presented in this study provide the most comprehensive evaluation to date of the possibility for oxygen supersaturation to improve the resilience of aquatic ectotherms to heatwaves.

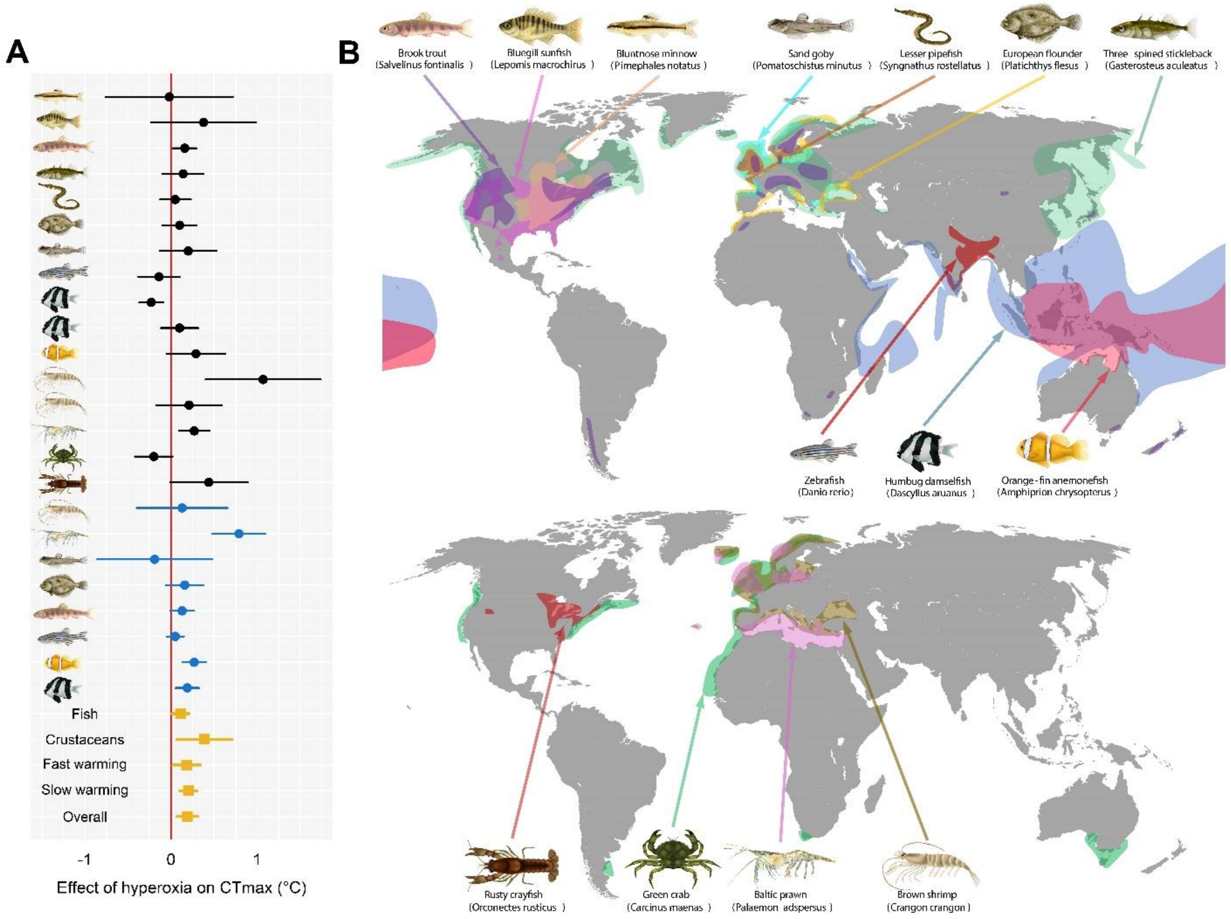

**Fig. 1. Effect of hyperoxia (150 % air saturation) on warming tolerance in 14 aquatic ectotherms from across the globe**. **A**: Forest plot showing effect sizes (model estimates ± 95% confidence intervals) for the effect of hyperoxia on warming tolerance. Black symbols are the fast warming (0.3°C min$^{-1}$) trials, blue symbols are the slow warming (1°C hour$^{-1}$) trials, and yellow symbols are for a net combined effect with random effects for subgroups of the 24 experiments. The effects were considered statistically significant where the 95% confidence interval does not cross the red vertical line (full statistics given in Supplementary Table S3). **B**: Approximate geographical distributions for the ten species of fish (top) and four species of decapod crustaceans (bottom) used in the laboratory experiments to assess the effects of hyperoxia on upper thermal tolerance (species distributions from aquamaps.org).

## Results

In the fast-warming experiments (0.3°C $min^{-1}$ warming rate), hyperoxia did not significantly increase warming tolerance ($CT_{max}$) in 12 of 14 species (Fig. 2 and Fig. 3, statistics in Supplementary Table S3). One exception was the brown shrimp *Crangon crangon* in 2022 (Fig. 3a), where hyperoxia increased warming tolerance by 1.06 ± 0.67°C (effect size; mean ± 95% confidence interval; $P = 0.002$). However, when we ran a second set of trials on brown shrimp in 2024, the effect did not occur ($P = 0.28$, Fig. 3a). In Baltic prawn, hyperoxia increased $CT_{max}$ by 0.27 ± 0.18°C ($P = 0.002$; Fig. 3d). Hyperoxia decreased $CT_{max}$ by 0.23 ± 0.14°C in humbug damselfish *Dascyllus aruanus* in our first experiment on the species in 2023 ($P = 0.002$; Fig. 2i), but the effect did not occur when we repeated the experiment in 2024 (0.10 ± 0.22°C, $P = 0.36$, Fig. 2i). In brook trout *Salvelinus fontinalis* there was a tendency for hyperoxia to increase warming tolerance by 0.16 ± 0.14°C ($P = 0.02$; Fig. 2c), but this did not reach our threshold for statistical significance ($\alpha = 0.01$; see *Materials and Methods*). Overall, when pooling the fast-warming experiments into a single model (with species-specific random intercepts), there was a negligible effect of hyperoxia (0.18 ± 0.16°C; $P = 0.02$; Fig. 1, Supplementary Table S3).

In the slow-warming experiments (1°C $h^{-1}$ warming rate), which we ran using 8 of 14 species, there was no effect of hyperoxia in 5 of the 8 species. In the orange-fin anemonefish *Amphiprion chrysopterus* there was a $CT_{max}$ increase of 0.27 ± 0.14°C (mean ± 95% CI) with hyperoxia ($P < 0.001$; Fig. 4g), while a hyperoxia-induced increase in $CT_{max}$ of the humbug damselfish was smaller (0.19 ± 0.14°C, $P = 0.007$, Fig. 4f). In Baltic prawn, the hyperoxia trial had a mean $CT_{max}$ that was 0.79 ± 0.31°C higher than the corresponding normoxia trial ($P < 0.001$, Fig. 4h). Notably, these slow-warming experiments had far fewer replicate animals and trials (typically one replicate trial per treatment) than did our fast-warming experiments, which typically had four replicate trials per treatment (Supplementary Table S2). The overall effect across all species was a tendency for a small increase of $CT_{max}$ with hyperoxia (0.20 ± 0.10°C) in the slow-warming experiments ($P < 0.001$) (Supplementary Table S3; Fig.

S1). Across crustaceans (fast and slow warming combined), the mean effect of hyperoxia was 0.39 ± 0.32°C ($P = 0.01$), and in fishes it was 0.11 ± 0.10°C ($P = 0.03$; Fig. 1, Supplementary Table S3).

Across our experiments (fast and slow warming combined), body mass had a positive effect on warming tolerance in five of the 24 experiments, and a negative effect in two experiments (Supplementary Table S3, Fig. S1). In most cases, any statistically significant effect of body mass that did arise was weak (Supplementary Fig. S1). Importantly, we did not find an interaction between oxygen saturation and body size in any of the experiments. In general, however, the range in body mass was low in each experiment because our study was not designed to assess the size-dependency of warming tolerance.

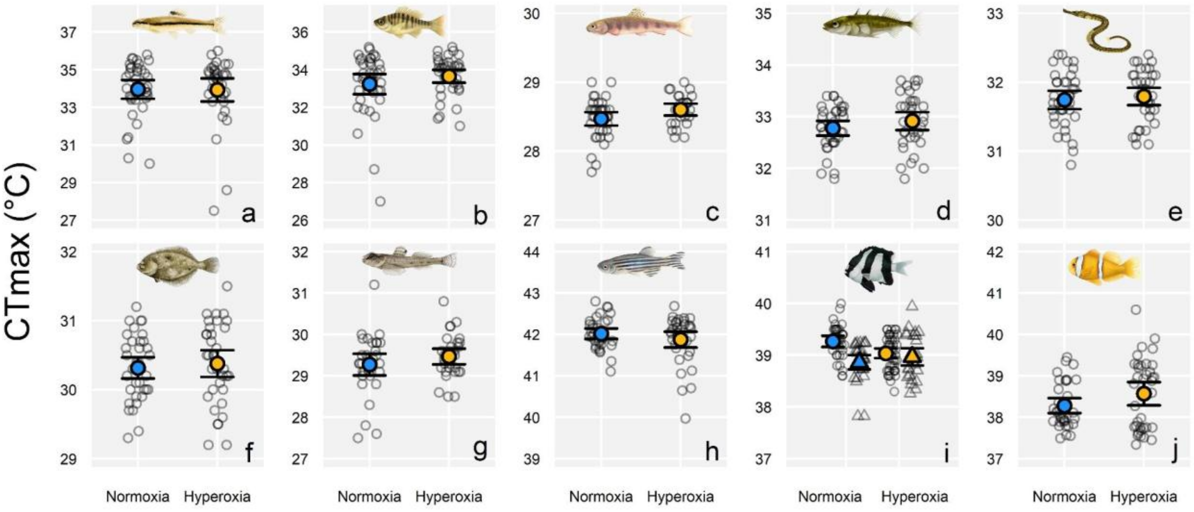

**Fig. 2. Tolerance to fast warming (0.3°C min$^{-1}$) under normoxia and hyperoxia in 10 tropical and temperate fishes.** Shown is the temperature at which loss of motor function occurred ($CT_{max}$) under normoxia (blue; 100% air saturation) and hyperoxia (yellow; *ca*. 150% air saturation). The large symbols show mean values, with individual raw data points scattered behind (error bar = 95% CI). Of these data, the only significant treatment effect ($P < 0.01$) was in the 2023 humbug damselfish (i) experiment in which there was a decrease in $CT_{max}$ under hyperoxia (Table S3). Species and sample sizes (*n* = normoxia, hyperoxia) are as follows: (a) bluntnose minnow *Pimephalus notatus* (35, 34), (b) bluegill *Lepomis macrochirus* (38, 37), (c) brook trout *Salvelinus fontinalis* (36, 26), (d) three-spined stickleback *Gasterosteus aculeatus* (35, 35), (e) lesser pipefish *Syngnathus rostellatus* (36, 35), (f) European flounder *Platichthys flesus* (36, 35), (g) sand goby *Pomatoschistus minutus* (31, 30), (h) zebrafish *Danio rerio* (34, 35), (i) humbug damselfish *Dascyllus aruanus* in 2023 (36, 46) (left - circles), and in 2024 (28, 26) (right - triangles), and (j) orange-fin anemonefish *Amphiprion chrysopterus* (36, 36).

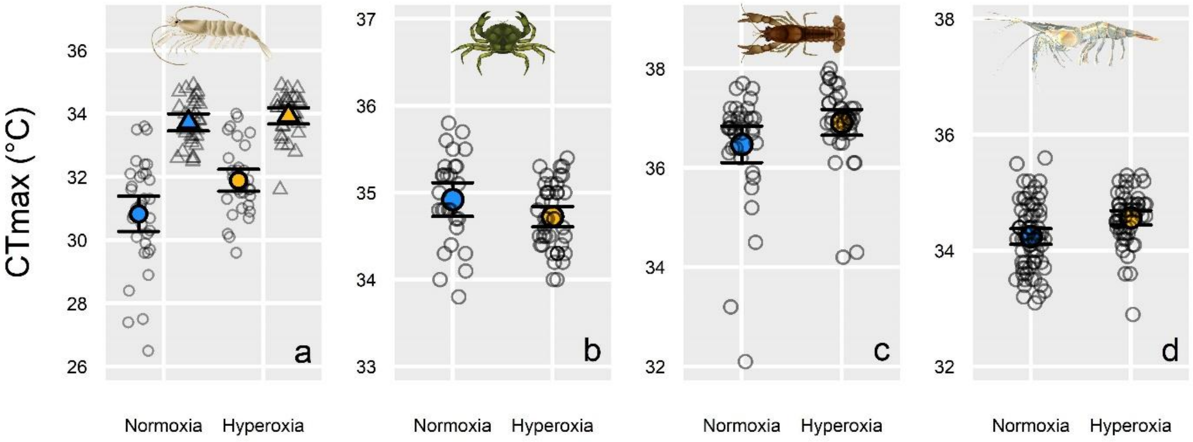

**Fig. 3. Tolerance to fast warming (0.3°C min$^{-1}$) under normoxia and hyperoxia in four temperate decapod crustaceans.** Shown is the temperature at which loss of motor function occurred (CT$_{max}$) under normoxia (blue; 100% air saturation) and hyperoxia (yellow; *ca*. 150% air saturation). The large symbols show mean values, with individual raw data points scattered behind (error bar = 95% CI). Species and sample sizes (*n* = normoxia, hyperoxia) are as follows: (a) left: brown shrimp *Crangon crangon* in 2022 (35, 35), right: 2024 (29, 30), (b) green crab *Carcinus maenas* (28, 42), (c) rusty crayfish *Ocronectes rusticus* (37, 37), (d) Baltic prawn *Palaeomon adspersus* (70, 53). The treatment difference was statistically significant for brown shrimp in 2022 ($P = 0.002$) but not in 2024 ($P = 0.28$). For Baltic prawn, the treatment effect was significant ($P = 0.003$; $P > 0.05$ for green crab and rusty crayfish; Supplementary Table S3).

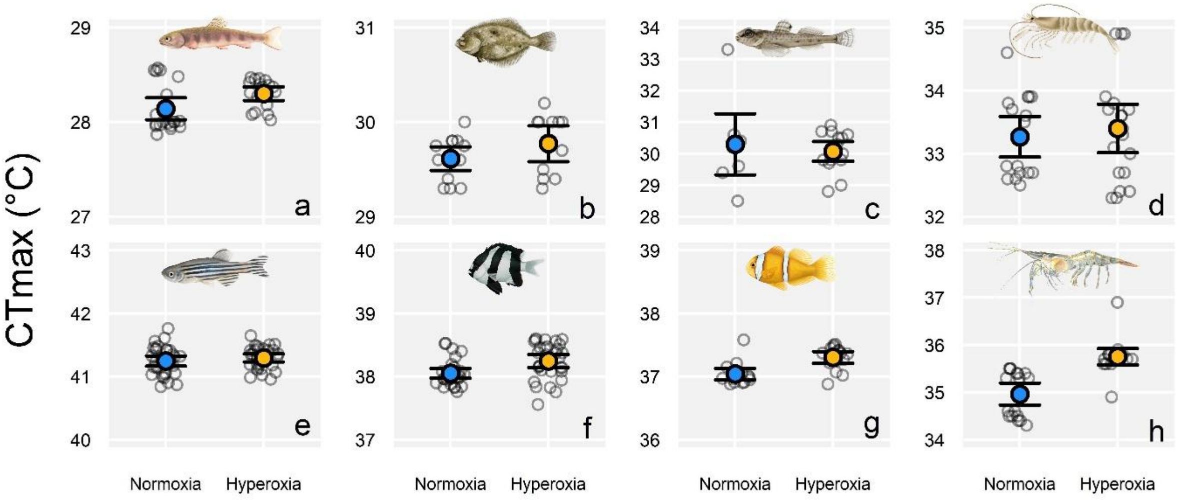

**Fig. 4. Tolerance to slow warming (1°C h$^{-1}$) under normoxia and hyperoxia in eight temperate and tropical ectotherms.** Shown is the CT$_{max}$ under normoxia (blue; 100% air saturation) and hyperoxia (yellow; *ca*. 150% air saturation). The large symbols show mean values, with individual raw data points scattered behind (error bar = 95% CI). Species and sample sizes (*n* = normoxia, hyperoxia) are as follows: (a) brook trout *Salvelinus fontinalis* (19, 17), (b) European flounder *Platichthys flesus* (13, 11), (c) sand goby *Pomatoschistus minutus* (8, 15), (d) brown shrimp *Crangon crangon* (16, 19), (e) zebrafish *Danio*

*rerio* (31, 29), (f) humbug damselfish *Dascyllus aruanus* (28, 30), (g) orange-fin anemonefish *Amphiprion chrysopterus* (15, 15), (h) Baltic prawn *Palaeomon adspersus* (17, 17). Zebrafish and humbug damselfish slow-warming trials involved two replicate $CT_{max}$ trials per treatment; all other species were based on a single slow-warming replicate trial per treatment. Treatment differences were statistically significant ($P < 0.01$) for humbug damselfish (f, $P = 0.007$), orange-fin anemonefish (g, $P < 0.001$), and in Baltic prawn (h, $P < 0.001$; statistics in Supplementary Table S3).

## Discussion

The data here provide the most comprehensive assessment to date of the effect of oxygen supersaturation on warming tolerance in aquatic ectotherms. For most of the species and heating rates (19 of the 24 experiments), hyperoxia did not increase warming tolerance, resulting in an estimated increase of 0.19 ± 0.12°C as the overall effect size across the study (Fig. 1). In 4 of the 14 species, we did see evidence for small increases in $CT_{max}$ under hyperoxia, particularly in the slow warming experiments. The largest effect size was in brown shrimp, which benefited from a *ca.* 1°C mean increase in $CT_{max}$ with hyperoxia during fast warming during our initial experiment on the species in 2022. However, when we repeated that experiment in 2024, the effect disappeared, suggesting it was a false positive or had some inexplicable context dependency. In Baltic prawn, orange-fin anenomefish, and humbug damselfish, minor effects of hyperoxia were detected (0.19 – 0.79°C increases in warming tolerance; Fig. 1). Collectively, our data suggest that the oxygen supersaturation that commonly occurs in shallow, productive aquatic ecosystems is unlikely to provide meaningful survival benefits for most ectotherms during heatwaves.

Unlike our findings, warming tolerance was reported to increase substantially in all six species under hyperoxia (140% air saturation) in 2°C $h^{-1}$ ramping rate experiments on ectotherms from the Red Sea [3], with increases ranging from 1.2 to 3.5°C. One of the species we tested, humbug damselfish *D. aruanus*, was also measured in that study and thus offers a point of direct comparison [3]. In our first experiment with humbug damselfish exposed to fast warming, we found that hyperoxia caused a small decrease (0.23°C) in warming tolerance, contrasting with the 1.8°C increase reported from the single warming tolerance trial per treatment conducted on *Dascyllus* sp. from the Red Sea [3]. Our second experiment on humbug damselfish the following year yielded no effect of hyperoxia in the fast-warming $CT_{max}$ trials, suggesting that the reduction in tolerance in the first experiment was a spurious result. We did see a small hyperoxia-induced improvement in $CT_{max}$ of 0.19°C in our slow-warming trial for this species, a fraction of the improvement of 1.8°C reported previously [3]. The difference in the effect of hyperoxia between our study and that of Giomi et al. [3] cannot be explained by differences in warming rate, as we used fast- and slow-warming rates that encompassed the warming rates used previously. One difference was that Giomi et al. [3] used median lethal time ($LT_{50}$; temperature at which 50% of animals died) instead of $CT_{max}$, checking on groups of animals (for mortality) every 30 minutes. While $LT_{50}$ differs from $CT_{max}$, it is generally accepted that death closely follows the $CT_{max}$ endpoint (i.e., seconds or minutes later, [15]) and therefore $LT_{50}$ and $CT_{max}$ should be broadly comparable. However, in their use of $LT_{50}$, Giomi et al. [3] only generated a single estimate of warming tolerance for each species and treatment, with no replicate trials (precluding the use of statistics). Modest variations in abiotic environmental factors other than temperature (e.g., salinity, dissolved $CO_2$, pH) typically have limited effects on warming tolerance in aquatic organisms, so these seem unlikely to be responsible for stark differences across studies [16–20]. Ultimately, we are not able to explain the differences between our study and that of Giomi et al. [3], but we are confident our estimates of the effects of hyperoxia are robust given the statistical power and replication in our study.

Most studies that have assessed the effect of hyperoxia on warming tolerance across tropical, temperate, and Antarctic fish species have either found no effect or a relatively small positive effect (typically <1°C, reviewed by [9]). However, of the previous studies that have found small increases in $CT_{max}$ in hyperoxia, many involved small sample sizes and a single warming tolerance trial per treatment. For tests of warming tolerance like $CT_{max}$, it is valuable to conduct multiple replicate trials per treatment to obtain accurate estimates of treatment effects. Our results show that even with multiple replicate trials (each with several animals), small, context-specific treatment differences (as we observed in a few instances) can disappear with further replication, as occurred here with humbug damselfish and brown shrimp. We ran four replicate $CT_{max}$ trials in most cases for the fast-warming experiments (sample sizes in Supplementary Table S2), providing a glimpse into inter-trial variability within treatments (Supplementary Fig. S3 and Table S4). Even with the same experimenter scoring $CT_{max}$ on the same species, we found that there was often a range of 0.5°C or more in mean $CT_{max}$ among replicate trials (7-10 animals per trial), with larger ranges in mean trial-specific $CT_{max}$ of 2.5–3°C in 2 of 13 species (brown shrimp and bluntnose minnow; Supplementary Fig. S3 and Table S4). Thus, a treatment effect for $CT_{max}$ (or $LT_{50}$) should be interpreted with caution if based on a single trial per treatment (or low sample sizes generally), especially if the effect size is small (e.g., 0.5°C or less), as has been the case in several previous studies on the effects of hyperoxia on warming tolerance and in some of the slow-warming experiments we conducted here.

Given the predictions of the oxygen-limitation hypothesis [7], directly removing any oxygen supply limitation via supersaturation can be an elegant way to experimentally assess the role of oxygen in warming tolerance [2]. Indeed, of the eighteen studies that have measured the partial pressure of oxygen in arterial blood ($PaO_2$) in fish acclimated for hours or days to hyperoxia, nearly all have found substantial increases in $PaO_2$ [5]. Of those studies that used environmental hyperoxia within the range of our study (*ca*. 125–175% air saturation), $PaO_2$ increased by a factor of *ca*. 1.5–2 in fish [5]. In turn, environmental hyperoxia can enable fish to increase their uptake of oxygen (i.e., maximum aerobic metabolic rate) and aerobic scope (i.e., the difference between standard and maximum aerobic metabolic rates) [21,22]. For example, Skeeles et al. [22] found a 74–95% increase in aerobic scope following acute (~ 4 h) exposure to hyperoxia (150% air saturation), while Brijs et al. [21] also reported close to a doubling of aerobic scope after 14 h of exposure to 200% air saturation. Based on these previous experiments, the animals in our study likely had higher oxygen availability in their tissues when tested in hyperoxia versus normoxia, yet warming tolerance was unaffected in most cases with the possible exception of the Baltic prawn.

Ultimately, our data suggest that the presence of oxygen supersaturation during heatwaves in temperate and tropical aquatic habitats is unlikely to improve the survival of most resident ectotherms. Oxygen is crucial to life and can affect the thermal performance and tolerance of water breathers under some contexts [2,5,10,23], especially under moderate or severe hypoxia [10,23,24]. However, with the new dataset presented here, we can conclude that incorporation of naturally occurring oxygen supersaturation into mechanistic species distribution models and thermal risk assessments is unlikely to improve their predictive ability [25–27]. Instead, incorporating a protective effect of hyperoxia into predictions could severely overestimate the resilience of marine animals to climate warming.

## Materials and Methods

### *Study sites, species, and holding conditions*

We used 14 species for this study (Fig. 1), 12 of which were wild animals we captured in the field and brought into the laboratory for experimentation. The first series of experiments, on temperate marine species, took place in 2022 at Kristineberg Marine Station (animal ethics permit #Dnr 5.8.18-8955/2022 issued to Jutfelt from the Ethical Committee for Animal Research in Gothenburg), Sweden, by the Gullmars Fjord, Skagerrak Sea (58.24965 N, 11.44585 E). We collected four marine fishes (sand goby *Pomatoschistus minutus*, three-spined stickleback *Gasterosteus aculeatus*, lesser pipefish *Syngnathus rostellatus*, European flounder *Platichthys flesus*) and two marine decapod crustaceans (brown shrimp *Crangon crangon*, green crab *Carcinus maenas*) by beach seine (1 × 8 m, 3 mm mesh) in shallow (<1 m) coastal environments that periodically exhibit hyperoxia (Supplementary Fig. S2). Animals were acclimated to the laboratory for at least 24 h before being used in $CT_{max}$ trials, in tanks supplied with constant flow-through of seawater supplied from the fjord (in normoxia, ambient temperatures, mean ± S.D. 16.26 ± 0.66°C for sand shrimp and green crab, 17.54 ± 0.97°C for the other species). Fish and decapods were fed once daily with freshly thawed mysid (Akvarie Teknik) and *Pandalus borealis* shrimp and newly hatched artemia to apparent satiation but were fasted for 16–24 hours prior to use in $CT_{max}$ trials.

The second set of experiments, on temperate freshwater species, took place in 2022 in the laboratory at Trent University (hereafter, Trent U), Canada (44.359499 N, 78.289008 W; animal ethics permit #28105 issued to Raby by the Trent U Animal Care Committee) with four freshwater species. Two species, (bluegill *Lepomis macrochirus* [young-of-year] and bluntnose minnow *Pimephalus notatus* [juveniles and adults]), were collected within 2 km of Trent U from the Otonabee River using a beach seine (15 × 1.5 m, 3 mm mesh). The same beach seine was used to collect rusty crayfish *Orconectes rusticus* (juveniles and adults) from a pond on the Trent U campus. The fourth species used for experiments at Trent U was brook trout *Salvelinus fontinalis* (juveniles), which were provided by the Codrington Fisheries Research Facility (Ontario Ministry of Natural Resources, 44.14760 N, 77.80190 W) after being incubated and raised (to ~6 months post-hatching) from the gametes of spawning fish caught in Salt Creek, ON (44.149889 N, 77.940750 W), in the autumn of 2021. A second group of brook trout (2 months post-hatch) were later brought from the same hatchery to Trent U for slow warming (1°C $h^{-1}$) $CT_{max}$ trials in spring of 2023. Each of these species were fed daily with blood worms and/or commercial pellets but left unfed on the day they were tested, with tests generally commencing 1–2 days after fish arrived in the laboratory. At Trent U, animals were held in tanks which were continuously refreshed with water from the Otonabee River that was sand filtered and disinfected with an ozonation system. Each tank was also aerated with an air stone and further filtered with an aquarium canister filter. The tanks were thermostatically controlled to maintain a stable temperature matching (within *ca*. 2°C) the temperature at which fish were collected (rusty crayfish mean ± S.D. = 18.21 ± 0.69°C; bluntnose minnow = 21.24 ± 0.24°C; bluegill = 18.31 ± 0.51°C; brook trout = 8.25 ± 0.36°C).

The third set of experiments, on a tropical marine species, took place at CRIOBE research station in Moorea, French Polynesia, in 2023 (Ethical approval was granted from The Ministere de l'Agriculture et des Ressources marines, en charge de l'Alimentation et de la Recherche, et de la Cause animale (MPR) permit number 7445/MPR/DRM). Humbug damselfish *Dascyllus aruanus* (juveniles and adults) were collected while snorkeling in shallow coral reefs at Papetō'ai, northern Moorea. The fish were then quickly transported to holding tanks (100 L) where they were kept for one week prior to the experiments. Both collection site temperatures and holding tank temperatures were 28–29°C. The tanks had continuous flow through seawater and fish were fed dry feed daily, except in the last 24 h prior to the experiments.

The fourth set of experiments used zebrafish *Danio rerio*, a tropical freshwater species, in the laboratory at the Norwegian University of Science and Technology (NTNU) (63.4189015 N, 10.4026598 W; animal ethics permit #29878 issued to Jutfelt by the Norwegian Food Safety Authority) in 2023. The zebrafish were 8th generation offspring from wild fish collected in Northwest Bengal, India in 2016 [28]. The fish had been acclimated to a constant temperature of 28°C for a year prior to the $CT_{max}$ trials. Each holding tank (60 × 35 × 30 cm) was aerated using an air stone and contained a sponge filter and had a low rate of continuous water replacement. All individuals were fed twice every day with commercial flakes (TetraPRO Energy Multi-Crisp) but were fasted on the day of $CT_{max}$ trials.

The fifth set of experiments, on two tropical marine species, took place again at CRIOBE research station in Moorea, but in 2024 (Ethical approval was granted from The Animal Ethics Committee, Centre National de la Recherche Scientifique; permit number 006725). Humbug damselfish (juveniles and adults) were collected while scuba diving in shallow coral reefs (*ca*. 2 m depth) at different locations on the North coast of Moorea. Upon collection, fish were quickly transported to holding tanks (100 L) where they were allowed to acclimate for a minimum of one week prior to experiments. Orange-fin anemonefish, *Amphiprion chrysopterus* (juveniles), were obtained from Coopérative des Aquaculteurs de Polynésie Française (C.A.P.F.) at Tahiti, and transported to CRIOBE research station in Moorea, where they arrived in March 2024 and were quickly transferred to their holding tanks (100L). Holding tank temperatures ranged between 29 and 31°C. The tanks had continuous flow through seawater and fish were fed live *Artemia spp.*, except in the last 24 hours prior to the experiments.

The sixth and final set of experiments, on temperate marine species, took place at Kristineberg Marine Station (animal ethics permit #Dnr 5.8.18-07417/2024 issued to Jutfelt from the Ethical Committee for Animal Research in Gothenburg) in 2024. Two marine decapod crustaceans (brown shrimp and Baltic prawn *Palaemon adspersus*) were collected via beach seine in shallow coastal environments. Animal acclimation and holding were similar to our first set of experiments at the same location in 2022. The mean acclimation temperatures ± S.D. in holding tanks were 18.3 ± 0.63°C for brown shrimp and 18.42 ± 0.54°C for Baltic prawn. Decapods were fed once daily with thawed *Pandalus borealis* shrimp and were fasted the day of $CT_{max}$ trials. The animals for these experiments were held in the laboratory for at least 24 h (up to 5 days) prior to use in $CT_{max}$ trials.

### *Measurement of critical thermal maximum ($CT_{max}$)*

For all 14 species, we followed a standardized method for $CT_{max}$, with a warming rate of 0.3°C $min^{-1}$ [29]. In 8 of the 14 species (sand goby, European flounder, brook trout, zebrafish, orange-fin anemonefish, humbug damselfish, brown shrimp, Baltic prawn), we conducted additional $CT_{max}$ trials with a warming rate of 1°C $h^{-1}$. Animals were placed into the arena to acclimate for 30 minutes before warming began (at either normoxia [100% air saturation] or hyperoxia [150%], matching their holding acclimation temperature), except for the 2024 experiments with Baltic prawn and brown shrimp, which were given 10 minutes of arena acclimation. Heaters were then switched on, achieving a warming rate of 0.3°C $min^{-1}$ (or 1°C $h^{-1}$), with identical water volume and heating power used for all trials for a given species such that warming rates were consistent among replicate trials (photos of $CT_{max}$ arenas we used in Supplementary Fig. S4). Raw data for temperature and oxygen from our $CT_{max}$ trials are plotted in a supplementary file available on figshare: https://figshare.com/s/8d73d800d71de07a6696. We conducted 3–5 $CT_{max}$ trials per species and oxygen treatment (normoxia and hyperoxia), with $n$ = 7–10 animals per trial to achieve sample sizes of $n$ ~ 35 per oxygen treatment and species in most cases, and one or two trials per treatment (and species) for the slow-warming experiments (sample sizes in Supplementary Table S2). For the normoxia treatment, aeration with an air stone ensured the arena stayed close to 100% air saturation (typically 95–105%). For the hyperoxia treatment, a similar air stone connected to a cylinder of compressed $O_2$ was used to bubble $O_2$ into the arena until dissolved oxygen (DO) reached ~150% air saturation. DO was then monitored carefully, with regular adjustments to ensure DO remained within ~5% of 150%. To monitor and record DO and temperature for experiments at Kristineberg (2022) and Trent U, we used a YSI ProSolo ODO Optical Dissolved Oxygen Meter (https://www.ysi.com/prosolo-odo), with the meter set to log DO and temperature at 30 second intervals. For all other experiments we used a PyroScience Firesting-$O_2$ Optical Oxygen and Temperature Meter (https://www.pyroscience.com/) (recording rate of 1 Hz). For most of the trials at Trent U and Kristineberg (2022), we also logged temperature in the $CT_{max}$ arena using an RBR ProSolo Temperature logger (https://rbr-global.com/) set to log temperature every 10 s.

$CT_{max}$ was quantified as the temperature at which each animal lost equilibrium (i.e., righting reflex). Because we studied a diversity of organisms, these endpoints differed slightly in the way they were assessed among species. For most fishes, loss of equilibrium (LOE) was defined as the point where they could not maintain a stable upright position for three continuous seconds [30]. For the three decapod crustaceans, $CT_{max}$ was typically preceded (immediately) by bursting up off the bottom of the arena then drifting back to the bottom with negative equilibrium. However, we also used a small dip net or plastic probe to frequently turn the invertebrates upside-down to check whether they maintained their righting reflex. For any given species, the same person scored $CT_{max}$ for all animals for both treatments, and that person was always blinded to temperature. That is, a second person monitored temperature and oxygen, and recorded the temperature at which each animal was removed from the arena (i.e., its $CT_{max}$ value). Animals were transferred into individual recovery containers following $CT_{max}$ and given at least 10 min to recover (to confirm they regained equilibrium and normal ventilation). Each animal was then euthanized with a lethal overdose of tricaine methanesulfonate (MS-222, Pharmaq) or clove oil (C8392, Sigma

Aldrich) before being weighed and measured, with the exception of the humbug damselfish in Moorea and decapods at Kristineberg Marine Station in 2024, which were released after being weighed, measured, and recovered overnight.

### *Statistics*

The effect of oxygen treatment on $CT_{max}$ was modeled separately for each species using linear models with body mass (log-transformed) as a covariate and an interaction between mass and oxygen treatment (normoxia, hyperoxia). The interaction was removed if it was not significant ($\alpha = 0.05$). Likewise, if mass had no effect on $CT_{max}$ ($\alpha = 0.05$), it was removed from the model. We tested for the effect of hyperoxia on $CT_{max}$ in 14 species for the fast-warming trials (0.3°C $min^{-1}$, including two separate models for two sets of humbug damselfish experiments), and separately for slow-warming trials (8 of 14 species, 1°C $h^{-1}$), for 24 models in total (linear models). In addition, to generate an overall effect size estimate (i.e., aggregating all 1451 data points), we ran a linear mixed effects (using the 'lme' function from the 'nlme' package in R[31]) model using oxygen treatment as a fixed effect and experiment (i.e., each species × warming rate combination) as a random effect (random intercept and random slope, i.e., "random = ~1 + oxygen treatment | experiment ID" allowing slopes and intercepts to vary for the 24 experiments). We used the same mixed effects model approach to generate effect-size estimates for fish, crustaceans, slow warming experiments, and fast warming experiments as larger groups (i.e., in each case, experiment ID was used as a random effect, as above). In most cases with these group models, a random term using random slope and intercepts provided better fit than using only random intercepts (based on ΔAIC and log-likelihood tests). There were two exceptions: for the fish model and for the slow warming model, adding a random slope did not improve model fit (so only random intercept models were used). Given that we conducted 29 separate statistical tests (24 experiments + 5 aggregate tests of different subgroups) of the null hypothesis that hyperoxia does not affect warming tolerance ($CT_{max}$), we wished to guard against type I errors via an adjustment to our significance threshold (α). However, bonferonni corrections (dividing 0.05 by the number of tests, in our case 0.05/29 = 0.002) can be overly conservative [32], resulting in a high risk of type II errors. Thus, to strike a balance between avoiding type I and type II errors, we set α to an intermediate value of 0.01. However, recognizing that *P*-values can be viewed at as a continuum of the strength of evidence (rather than a binary test; [33], and that null hypothesis statistical testing has been criticized [34], we place emphasis on effect sizes in our interpretations. Model assumptions were assessed by visual inspection of residuals. Analyses were conducted using R (v.4.4.1 [35]) with RStudio (v.2024.09.0 [36]).

**Data and code availability** The raw data are archived on figshare: https://figshare.com/s/8e9d217bd494d0121fc1 (fast warming data), https://figshare.com/s/8e9d217bd494d0121fc1 (slow warming data); readme here: https://figshare.com/s/349131ec66331d118a56. Plots of oxygen concentration and temperature from $CT_{max}$ trials are available here: https://figshare.com/s/8d73d800d71de07a6696. The R code used for statistical analyses is available here: https://figshare.com/s/ff7e610970c1f8880edc. (Note: these are private links to unpublished figshare files: all files will be shared via a single public figshare link before publication.)

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**Acknowledgments** This study was funded by a European Research Council Consolidator grant (CLIMEVOLVE; to Jutfelt) and by the Natural Sciences and Engineering Research Council of Canada (Discovery Grants to Raby and to Speers-Roesch). Clark was supported by an Australian Research Council Future Fellowship (FT180100154) funded by the Australian Government. This project also benefitted from co-funding between the EU program for research and innovation Horizon Europe and Marie Skłodowska-Curie n°101081465 (AUFRANDE) to Storm and Mills and Pacific Funds "BLEACHALAN" and Recherche et Innovation Partenariat Public Privé RIP4 "Raising Nemo" to Mills. We thank the staff at Kristineberg Marine Research Station and the Animal Care team at Trent University for logistical support. Chris Wilson and Vince Frasca (Ontario Ministry of Natural Resources) provided the brook trout we used in this study. Assistance with animal collections, husbandry, and experimentation was provided by Natalie Sopinka, Hanna Scheuffele, Josefin Sundin, Eirik Åsheim, Andrea Campos Candela, Pieter Riesenkamp, Martin Henriksson, Sienna Overduin, Rob Griffin, Tamzin Blewett, Sandra

Binning, Lauren Rowsey, Christian Bihun, Jacob Bowman, Erin Ritchie, Nathan Obach, Leah Howitt, Amanda Reynolds, Jordie Keary, Jules Schligler, Shamil Debaere, Marie Levet, and Eline Rypdal. We thank the FIN club workshops in 2022 and 2023 for providing the opportunity to conduct the experiments, analyses, and writing. Victoria Thelamon created most of the animal illustrations; the Baltic prawn illustration was by Chris Macleod. The RBR temperature loggers we used were provided by the Real Time Aquatic Ecosystem Observation Network (University of Windsor, Canada; www.raeon.org).

**Author contributions** Designed the experiment: G.D.R, T.D.C., F.J.. Data collection: all authors. Data analysis: G.D.R. Instruments, materials, and funding: G.D.R, B.S.R., S.M., T.D.C., F.J.. Writing: G.D.R. with input from all co-authors.

# Supplementary Information for

*Oxygen supersaturation has negligible effects on warming tolerance in aquatic ectotherms*

**Table S1.** The range of oxygen supersaturation that occurs in the ecosystems relevant to the species included in our study. Hyperoxia (dissolved oxygen partial pressures >100% air saturation) in the wild is evident from several studies from the early '90s to early 2020's. In general, the phenomenon occurs when primary producers release oxygen from photosynthesis into water and warming simultaneously decreases the water's oxygen solubility (Giomi et al. 2019). Aquatic ecosystems with a high proportion of primary producers relative to respiring animal biomass, easy access of sunlight due to shallow depth and limited water exchange can become saturated with oxygen, and a relative increase in temperature will therefore supersaturate the water, even at temperatures that might not be perceived as "warm". The time of the day when the water heats up the fastest also varies depending on the ecosystem. For example, midday is reported in the tropics, where a zenithal sun position provides the strongest energy input (Giomi et al. 2019). In contrast, late afternoon can be the warmest time in the northern hemisphere, where a colder climate and lower angle of the sun slows down heat transfer and creates a lag. Heating rate is further affected by how isolated the water is and can thus be influenced by tidal cycles in closed-off bays, lagoons, tidal marshes and rock pools.

| Habitat type and location | [DO] (% air saturation) | Temp (°C) | Salinity (ppt) | Depth (m) | Relevant species | Source |
|---|---|---|---|---|---|---|
| Near shore pelagic, Southern Baltic Sea | 107-132 | 12-17 | 7-12* | <1 | Lesser pipefish, Three-spine stickleback, Sand goby, European flounder, Green crab, Brown shrimp | Marks (2008).<br>https://doi.org/10.2166/nh.2008.021 |
| Pelagic, Skagerrak Baltic Sea | 102-115.7 | 12-17 | 7-31 | <15 | Three-spine stickleback, Sand goby, European flounder, Green crab, Brown shrimp | Stigebrandt (1991).<br>https://doi.org/10.4319/lo.1991.36.3.0444 |
| Experimental shallow soft bottom community, Baltic Sea | 134 | 18 | 7 | NA | Sand goby, European flounder, Green crab, Brown shrimp | Gorska et al. (2018).<br>https://doi.org/10.1016/j.jmarsys.2018.01.001 |
| Pelagic, Baltic Sea | 133-152 | NA | 5-8.6 | <15 | Three-spine stickleback | Rahm et al. (1995).<br>https://doi.org/10.1007/BF00552572 |
| Pelagic, North Sea | 124-188 | 15-18* | 33-35* | <4 | Larvae of Sand goby, European flounder, Green crab, Brown shrimp | Riebesell (1992).<br>https://doi.org/10.4319/lo.1992.37.1.0063 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Surface waters in tidal channels, Wadden Sea | 100-148 | 12-15 | 19-28 | <19 | Lesser pipefish, Three-spine stickleback, Sand goby, European flounder, Green crab, Brown shrimp | Hoppema (1991).<br>https://doi.org/10.1016/0272-7714(91)90036-B |
| Large river, Grand River watershed, Ontario, Canada | 150-180 | 20 | 0* | 0.5 - 1 | Brook trout, Bluegill sunfish, Blunthead minnow, rusty crayfish | Rosamond et al. (2011).<br>https://doi.org/10.2134/jeq2010.0009 |
| Meltwater influenced lake, Kootenay Lake, British Columbia, Canada | 121-140 | NA | 0* | 2 | Brook trout, Bluegill sunfish | Northcote et al. (2005).<br>doi.org/10.1080/07438140509354434 |
| Mangrove forest, Red Sea, Saudi Arabia | 100-250 | 28-42 | 42 | NA | Humbug damselfish | Giomi et al. (2019).<br>doi.org/10.1126/sciadv.aax1814 |
| Large river, Kanhan River, Vidharba, India | 151 | 28 | 0* | NA | Zebrafish | Central Water Commission (2019).<br>https://cwc.gov.in/sites/default/files/effect-time-and-temperature-do-levels-river-water-2019.pdf |

*not specified or recorded in the study, but retrieved from other regional environmental data.

**Table S2.** Sample sizes and body mass for each of the 24 sets of $CT_{max}$ experiments for this study. Fast warming = 0.3°C $min^{-1}$, slow warming = 1°C $h^{-1}$.

| **Species** | **Treatment** | **Warming rate** | **Body mass, g (mean, range)** | **Trials (*n*)** | **Animals (total *n* across trials)** |
|---|---|---|---|---|---|
| bluntnose minnow, *Pimephalus notatus* | hyperoxia | fast | 1.17 (0.22 – 6.96) | 4 | 34 |
| | normoxia | fast | 1.21 (0.40 – 8.29) | 4 | 35 |
| brook trout, *Salvelinus fontinalis* | hyperoxia | fast | 5.39 (2.82 – 10.80) | 3 | 26 |
| | normoxia | fast | 6.00 (3.55 – 12.54) | 4 | 36 |
| rusty crayfish, *Orconectes rusticus* | hyperoxia | fast | 4.86 (0.75 – 29.75) | 4 | 37 |
| | normoxia | fast | 3.27 (0.25 – 9.79) | 4 | 37 |
| bluegill sunfish, *Lepomis macrochirus* | hyperoxia | fast | 0.74 (0.25 – 1.74) | 4 | 37 |
| | normoxia | fast | 0.71 (0.16 – 1.49) | 4 | 38 |
| European flounder, *Platichthys flesus* | hyperoxia | fast | 0.58 (0.28 – 1.78) | 4 | 36 |
| | normoxia | fast | 0.61 (0.11 – 1.72) | 4 | 36 |
| green crab, *Carcinus maenas* | hyperoxia | fast | 1.57 (0.31 – 3.65) | 4 | 42 |
| | normoxia | fast | 1.50 (0.31 – 3.38) | 3 | 28 |
| humbug damselfish, *Dascyllus aruanus* | hyperoxia | fast | 1.34 (0.13 – 4.12) | 5 | 46 |
| | normoxia | fast | 1.50 (0.08 – 5.05) | 4 | 36 |
| humbug damselfish experiment 2 | hyperoxia | fast | 1.23 (0.03 – 6.25) | 3 | 26 |
| | normoxia | fast | 0.82 (0.03 – 3.56) | 3 | 28 |
| orange-fin anenomefish, *Amphiprion chrysopterus* | hyperoxia | fast | 1.15 (0.60 – 1.86) | 4 | 36 |
| | normoxia | fast | 1.19 (0.58 – 2.24) | 4 | 36 |
| lesser pipefish, *Syngnathus rostellatus* | hyperoxia | fast | 0.65 (0.24 – 1.19) | 4 | 35 |
| | normoxia | fast | 0.70 (0.32 – 1.27) | 4 | 36 |
| sand goby, *Pomatoschistus minutus* | hyperoxia | fast | 1.82 (0.81 – 3.12) | 4 | 30 |
| | normoxia | fast | 1.60 (0.65 – 2.68) | 4 | 31 |
| brown shrimp, *Crangon crangon* | hyperoxia | fast | 0.70 (0.11 – 1.52) | 4 | 35 |
| | normoxia | fast | 0.76 (0.07 – 1.90) | 4 | 35 |
| brown shrimp experiment 2 | hyperoxia | fast | 0.49 (0.27 – 0.87) | 3 | 30 |
| | normoxia | fast | 0.56 (0.19 – 1.47) | 3 | 29 |
| Baltic prawn, *Palaemon adspersus* | hyperoxia | fast | 1.24 (0.33 – 3.24) | 5 | 53 |
| | normoxia | fast | 1.42 (0.37 – 3.87) | 7 | 70 |
| three-spined stickleback, *Gasterosteus aculeatus* | hyperoxia | fast | 1.42 (0.86 – 2.39) | 4 | 35 |
| | normoxia | fast | 1.81 (0.92 – 2.85) | 4 | 35 |
| zebrafish, *Danio rerio* | hyperoxia | fast | 0.25 (0.18 – 0.38) | 5 | 35 |
| | normoxia | fast | 0.25 (0.14 – 0.42) | 5 | 34 |

| | | | | | |
|---|---|---|---|---|---|
| brook trout, *Salvelinus fontinalis* | hyperoxia | slow | 0.60 (0.46 – 0.77) | 1 | 17 |
| | normoxia | slow | 0.57 (0.40 – 0.85) | 1 | 19 |
| European flounder, *Platichthys flesus* | hyperoxia | slow | 0.99 (0.43 – 3.15) | 1 | 11 |
| | normoxia | slow | 0.80 (0.45 – 2.15) | 1 | 13 |
| sand goby, *Pomatoschistus minutus* | hyperoxia | slow | 1.58 (1.31 – 2.18) | 1 | 15 |
| | normoxia | slow | 1.68 (0.71 – 2.57) | 1 | 8 |
| zebrafish, *Danio rerio* | hyperoxia | slow | 0.26 (0.12 – 0.39) | 2 | 29 |
| | normoxia | slow | 0.25 (0.11 – 0.43) | 2 | 31 |
| Humbug damselfish, *Dascyllus aruanus* | hyperoxia | slow | 1.32 (0.13 – 5.63) | 2 | 30 |
| | normoxia | slow | 1.41 (0.13 – 4.57) | 2 | 28 |
| orange-fin anenomefish, *Amphiprion chrysopterus* | hyperoxia | slow | 1.06 (0.59 – 2.46) | 1 | 15 |
| | normoxia | slow | 1.26 (0.64 – 2.46) | 1 | 15 |
| brown shrimp | hyperoxia | slow | 0.68 (0.24 – 1.24) | 1 | 19 |
| | normoxia | slow | 0.58 (0.38 – 0.88) | 1 | 16 |
| Baltic prawn | hyperoxia | slow | 1.04 (0.53 – 1.78) | 1 | 17 |
| | normoxia | slow | 1.66 (0.57 – 3.15) | 1 | 17 |
| | | | **Total** | **147** | **1451** |

**Table S3.** Model estimates for normoxia (intercept) and for the effects of hyperoxia for each of the 24 experiments modeled with separate linear models for each species. The mass covariate (log transformed) was removed if not significant (*P* > 0.05) in the final model, but we give the mass coefficient estimate and *P* values from the full model in those cases where it was not significant. The bottom five models are based on linear mixed effects models with random intercepts and slopes, except for the 'fish' model and the 'slow warming' model which were fit better using random intercepts only (based on comparison of AIC values and log-likelihood tests).

| **Species** | **Rate of warming (°C/hour)** | **Intercept (Warming tolerance at normoxia,° C) ± S.E.** | **Treatment coefficient (effect of hyperoxia, ° C) ± S.E.** | ***P*** | **Coefficient: log10 mass (g) ± S.E.** | ***P*** |
|---|---|---|---|---|---|---|
| bluntnose minnow | 18 | 34.04 ± 0.27 | -0.02 ± 0.38 | 0.96 | 1.82 ± 0.69 | 0.01 |
| bluegill | 18 | 33.68 ± 0.25 | 0.38 ± 0.31 | 0.22 | 2.03 ± 0.62 | 0.002 |
| brook trout | 18 | 27.94 ± 0.17 | 0.16 ± 0.07 | 0.02 | 0.71 ± 0.22 | 0.002 |
| three-spined stickleback | 18 | 32.78 ± 0.08 | 0.14 ± 0.12 | 0.237 | -0.61 ± 0.47 | 0.19 |
| lesser pipefish | 18 | 31.74 ± 0.07 | 0.05 ± 0.09 | 0.60 | -0.31 ± 0.30 | 0.31 |
| European flounder | 18 | 30.73 ± 0.10 | 0.10 ± 0.10 | 0.35 | 1.55 ± 0.25 | <0.001 |
| sand goby | 18 | 29.27 ± 0.12 | 0.20 ± 0.17 | 0.24 | -1.05 ± 0.54 | 0.06 |
| zebrafish | 18 | 42.02 ± 0.09 | -0.14 ± 0.12 | 0.24 | 1.06 ± 0.55 | 0.06 |
| humbug damselfish (2023) | 18 | 39.27 ± 0.05 | -0.23 ± 0.07 | 0.002 | 0.10 ± 0.10 | 0.31 |
| humbug damselfish experiment 2 (2024) | 18 | 38.88 ± 0.08 | 0.10 ± 0.11 | 0.36 | 0.05 ± 0.10 | 0.66 |
| orange-fin anemonefish | 18 | 38.30 ± 0.13 | 0.29 ± 0.17 | 0.10 | -0.45 ± 0.58 | 0.44 |
| brown shrimp (2022) | 18 | 30.83 ± 0.24 | 1.06 ± 0.34 | 0.002 | -0.63 ± 0.48 | 0.19 |
| brown shrimp experiment 2 (2024) | 18 | 33.72 ± 0.14 | 0.21 ± 0.19 | 0.28 | -0.70 ± 0.69 | 0.31 |
| Baltic prawn | 18 | 34.34 ± 0.06 | 0.27 ± 0.09 | 0.003 | -0.95 ± 0.22 | <0.001 |
| green crab | 18 | 34.92 ± 0.08 | -0.20 ± 0.11 | 0.07 | 0.36 ± 0.21 | 0.10 |
| rusty crayfish | 18 | 36.47 ± 0.16 | 0.44 ± 0.23 | 0.06 | -0.45 ± 0.30 | 0.14 |
| sand goby | 1 | 31.19 ± 0.38 | -0.19 ± 0.34 | 0.58 | -4.71 ± 1.40 | 0.003 |
| European flounder | 1 | 29.62 ± 0.08 | 0.16 ± 0.11 | 0.18 | 0.41 ± 0.25 | 0.11 |
| brook trout | 1 | 28.41 ± 0.11 | 0.16 ± 0.07 | 0.06 | 1.06 ± 0.37 | 0.008 |
| zebrafish | 1 | 41.25 ± 0.04 | 0.05 ± 0.05 | 0.36 | -0.09 ± 0.21 | 0.65 |
| clownfish | 1 | 37.04 ± 0.05 | 0.27 ± 0.07 | <0.001 | 0.07 ± 0.20 | 0.73 |
| humbug damselfish | 1 | 38.05 ± 0.05 | 0.19 ± 0.07 | 0.007 | -0.05 ± 0.08 | 0.46 |
| brown shrimp | 1 | 33.27 ± 0.19 | 0.13 ± 0.26 | 0.62 | -1.43 ± 0.93 | 0.13 |
| Baltic prawn | 1 | 34.97 ± 0.10 | 0.79 ± 0.15 | <0.001 | 0.08 ± 0.47 | 0.87 |
| Fish | 1 and 18 | 34.27 ± 1.32 | 0.11 ± 0.05 | 0.03 | | |
| Crustaceans | 1 and 18 | 34.06 ± 0.67 | 0.39 ± 0.16 | 0.01 | | |
| Fast warming | 18 | 34.27 ± 0.97 | 0.18 ± 0.08 | 0.02 | | |
| Slow warming | 1 | 34.07 ± 1.63 | 0.20 ± 0.05 | <0.001 | | |
| Overall | 1 and 18 | 34.20 ± 0.82 | 0.19 ± 0.06 | 0.002 | | |

**Table S4.** Statistics describing variation in $CT_{max}$ among fast-warming (0.3°C $min^{-1}$) replicate trials within a species and treatment (3-5 replicate trials per group, *ca*. 7-10 animals per replicate, see Table S2 for sample sizes). The *F* and *P* values are from ANOVAs testing for differences among replicate $CT_{max}$ trials. The $CT_{max}$ mean range refers to the difference between the highest and lowest mean within-trial $CT_{max}$ value. The data are visualized in Fig. S3.

| **Species** | **Treatment** | ***F* value** | ***P* value** | **Lowest mean $CT_{max}$** | **Highest mean $CT_{max}$** | **$CT_{max}$ mean range (°C)** |
|---|---|---|---|---|---|---|
| **bluntnose minnow** | hyperoxia | 5.75 | 0 | 32.2 | 34.9 | 2.68 |
| **bluntnose minnow** | normoxia | 15.82 | 0 | 32.3 | 35.2 | 2.92 |
| **brook trout** | hyperoxia | 0.09 | 0.91 | 28.6 | 28.6 | 0.05 |
| **brook trout** | normoxia | 0.79 | 0.51 | 28.4 | 28.6 | 0.19 |
| **orange-fin anemonefish** | hyperoxia | 3.55 | 0.03 | 37.9 | 39.0 | 1.10 |
| **orange-fin anemonefish** | normoxia | 3.34 | 0.03 | 38.0 | 38.7 | 0.71 |
| **rusty crayfish** | hyperoxia | 0.56 | 0.64 | 36.7 | 37.1 | 0.44 |
| **rusty crayfish** | normoxia | 1.31 | 0.29 | 36.1 | 37.0 | 0.89 |
| **European flounder** | hyperoxia | 10.77 | 0 | 29.7 | 30.9 | 1.14 |
| **European flounder** | normoxia | 1.35 | 0.27 | 30.2 | 30.6 | 0.39 |
| **green crab** | hyperoxia | 0.52 | 0.67 | 34.6 | 34.8 | 0.17 |
| **green crab** | normoxia | 13.56 | 0 | 34.3 | 35.2 | 0.85 |
| **humbug damselfish experiment 1 (2023)** | hyperoxia | 0.50 | 0.73 | 38.9 | 39.2 | 0.23 |
| **humbug damselfish experiment 1(2023)** | normoxia | 2.45 | 0.08 | 39.1 | 39.5 | 0.39 |
| **humbug damselfish experiment 2 (2024)** | hyperoxia | 1.36 | 0.28 | 38.8 | 39.1 | 0.33 |
| **humbug damselfish experiment 2 (2024)** | normoxia | 1.23 | 0.31 | 38.8 | 39.0 | 0.24 |
| **bluegill** | hyperoxia | 1.15 | 0.34 | 33.2 | 34.1 | 0.91 |
| **bluegill** | normoxia | 0.69 | 0.57 | 32.7 | 33.6 | 0.94 |
| **lesser pipefish** | hyperoxia | 1.64 | 0.2 | 31.7 | 32.0 | 0.37 |
| **lesser pipefish** | normoxia | 4.59 | 0.01 | 31.4 | 32.0 | 0.56 |
| **Baltic prawn** | hyperoxia | 1.66 | 0.17 | 34.3 | 34.8 | 0.49 |
| **Baltic prawn** | normoxia | 20.6 | 0 | 33.6 | 34.9 | 1.38 |
| **sand goby** | hyperoxia | 9.41 | 0 | 28.8 | 29.9 | 1.11 |
| **sand goby** | normoxia | 1.07 | 0.38 | 29.0 | 29.5 | 0.52 |
| **brown shrimp experiment 1 (2022)** | hyperoxia | 21.9 | 0 | 30.9 | 33.1 | 2.23 |
| **brown shrimp experiment 1 (2022)** | normoxia | 12.6 | 0 | 29.4 | 32.5 | 3.16 |
| **brown shrimp experiment 2 (2024)** | hyperoxia | 3.09 | 0 | 33.5 | 34.3 | 0.74 |
| **brown shrimp experiment 2 (2024)** | normoxia | 13.4 | 0 | 33.0 | 34.3 | 1.27 |
| **threespine stickleback** | hyperoxia | 1.71 | 0.18 | 32.5 | 33.1 | 0.52 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **threespine stickleback** | normoxia | 0.93 | 0.44 | 32.7 | 33.0 | 0.35 |
| **zebrafish** | hyperoxia | 1.20 | 0.33 | 41.5 | 42.0 | 0.58 |
| **zebrafish** | normoxia | 2.27 | 0.09 | 41.8 | 42.2 | 0.45 |

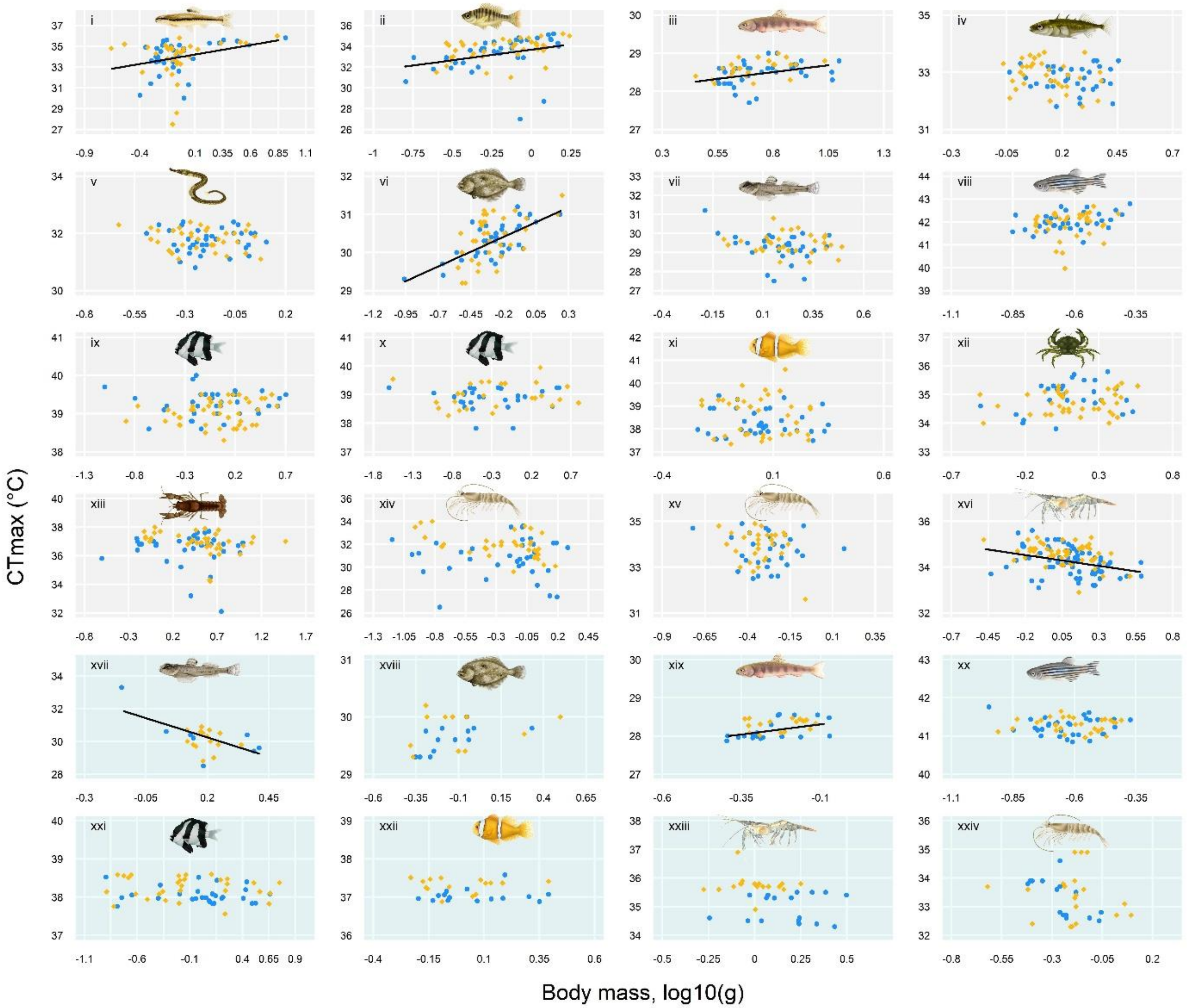

**Figure S1.** Temperature at which loss of motor function occurred ($CT_{max}$) in 24 experiments including 14 species of aquatic ectotherms, as a function of body mass ($log_{10}$-transformed, as in our statistics). Animals from the normoxia treatment are shown in blue circles, hyperoxia in yellow diamonds. Linear relationships are shown where they were statistically significant ($P < 0.01$, see Table S3). The 16 top panels are from the fast-warming trials (0.3°C $min^{-1}$), the bottom eight panels shaded in blue are the slow-warming (1°C $h^{-1}$) trials. The species are as follows: (i) bluntnose minnow *Pimephalus notatus*, (ii) bluegill *Lepomis macrochirus*, (iii) brook trout *Salvelinus fontinalis*, (iv) three-spined stickleback *Gasterosteus aculeatus*, (v) lesser pipefish *Syngnathus rostellatus* (vi), European flounder *Platichthys flesus* (vii), sand goby *Pomatoschistus minutus*, (viii) zebrafish *Danio rerio*, (ix) humbug damselfish *Dascyllus aruanus* experiment 1 (2023), (x) humbug damselfish experiment 2 (2024), (xi) orange-fin anenomefish *Amphiprion chrysopterus*, (xii) green crab *Carcinus maenas*, (xiii) rusty crayfish *Ocronectes rusticus*, (xiv) brown shrimp *Crangon crangon* experiment 1 (2022), (xv) brown shrimp *Crangon crangon* experiment 2 (2024), (xvi) Baltic prawn *Palaemon adspersus*, (xvii) sand goby, (xviii) European flounder, (xix) brook trout, (xx) zebrafish, (xxi) humbug damselfish, (xxii) orange-fin anenomefish, (xxiii) Baltic prawn, (xxiv) brown shrimp.

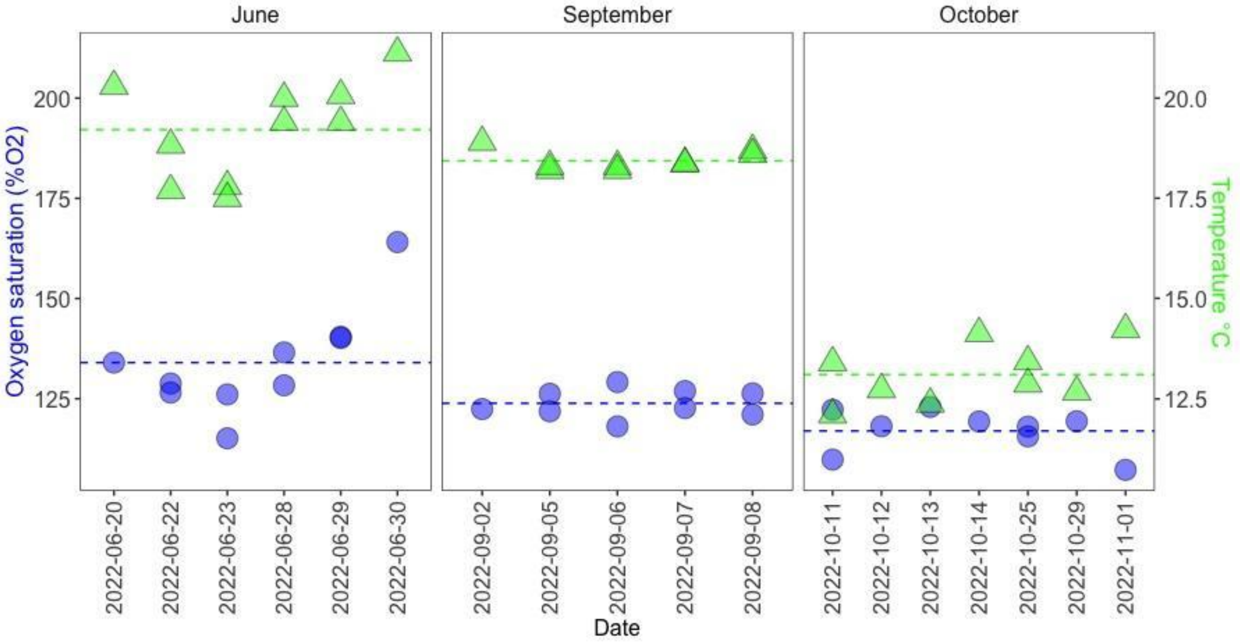

**Figure S2.** Measurements of supersaturation in the area where the following species were collected for the study's experiments: lesser pipefish *Syngnathus rostellatus*, three-spine stickleback *Gasterosteus aculeatus*, sand goby *Pomatoschistus minutus*, European flounder *Platichthys flesus*, green crab *Carcinus maenas* and brown shrimp *Crangon crangon*. In short, 9-10 seagrass *Zostera marina* meadows, where all the above listed species were found, within 10 km of Kristineberg Marine Station (58.24965 N, 11.44585 E), were sampled using a handheld oximeter at 1 m depth for temperature, salinity, and dissolved oxygen in June, September, and October 2022. The oxygen saturation point was then calculated using the o2.at.sat function in the LakeMetabolizer (Winslow et al., 2016, https://doi.org/10.1080/IW-6.4.883) package with the "garcia-benson" model applied to the data. From this, the oxygen saturation level of each site and date was calculated as $\%O_2$ = O2 / O2' x 100, where $O_2$ was the dissolved oxygen in the sample in mg $L^{-1}$ and $O_2$' was the oxygen solubility for each measurement of salinity and temperature. Blue circles show the calculated oxygen saturation. Green triangles show the corresponding temperature (shown on the right y-axis) measured at each site and date. Lines show the average value for all measurement points and month.

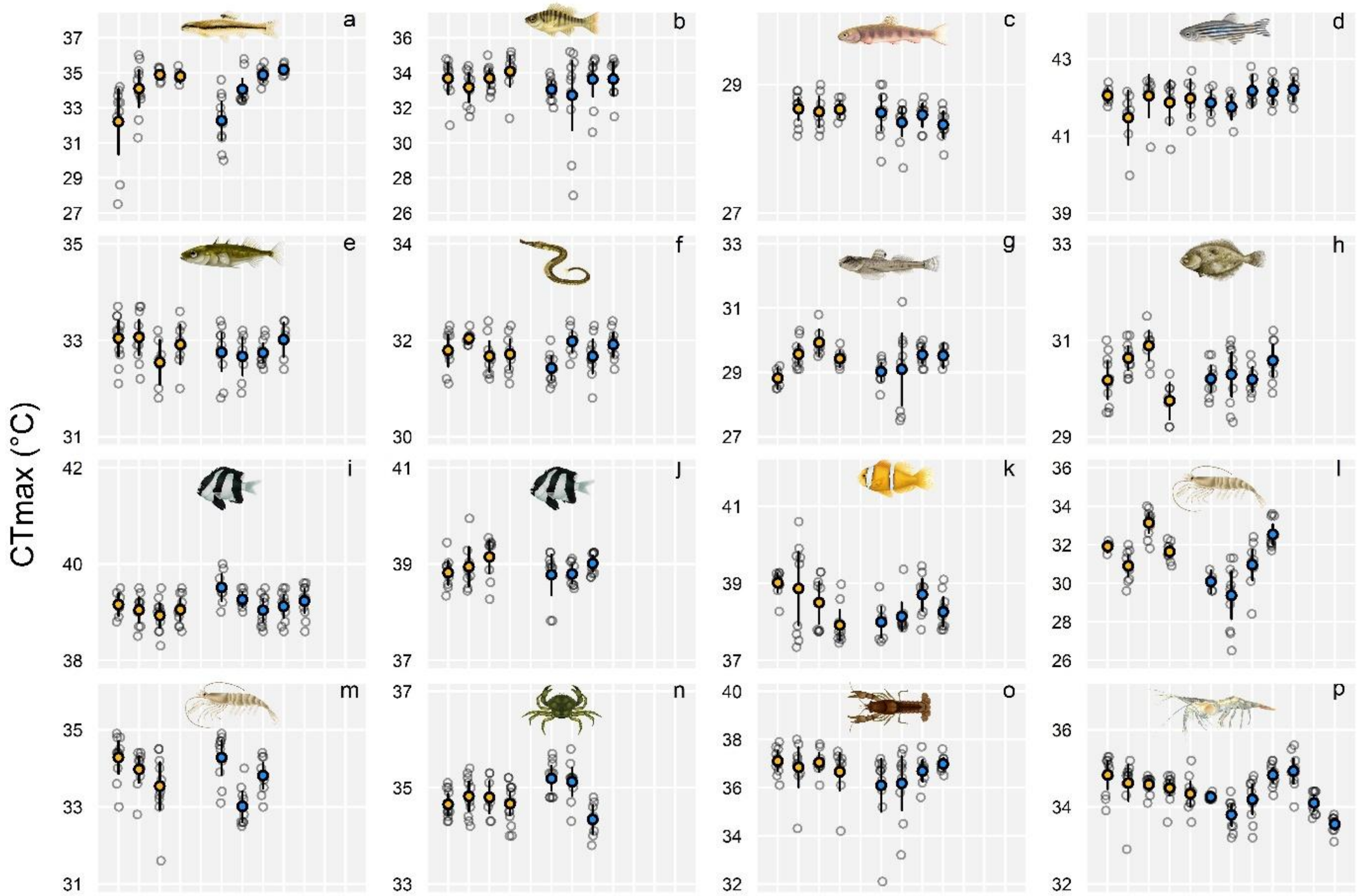

**Figure S3**. $\mathrm{CT}_{max}$ data for fast-warming (0.3°C $\mathrm{min}^{-1}$) plotted separately by replicate trials, with individual data points shown and mean (yellow = hyperoxia, blue = normoxia) and 95% confidence intervals plotted for each group. Sample sizes are given in table S2. a: bluntnose minnow, b: bluegill, c: brook trout, d: zebrafish, e: threespine stickleback, f: lesser pipefish, g: sand goby, h: European flounder, i: humbug damselfish experiment 1 (2023), j: humbug damselfish experiment 2 (2024), k: orange-fin anemonefish, l: brown shrimp experiment 1(2022), m: brown shrimp experiment 2 (2024), n: green crab, o: rusty crayfish, p: Baltic prawn. See Fig. S1 caption for scientific names.

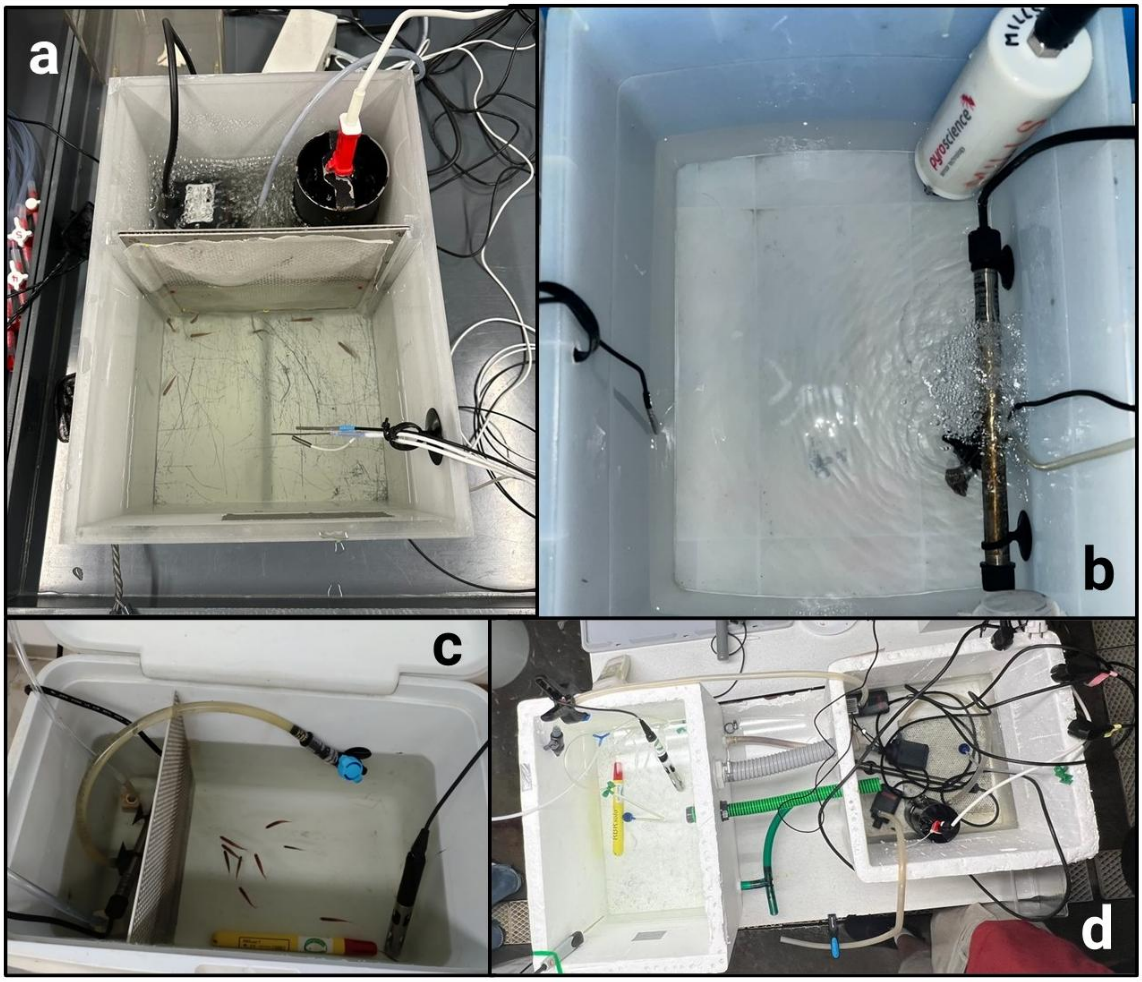

**Figure S4.** Overhead photos of $CT_{max}$ arenas we used. **a**: The arena we used for stickleback, zebrafish, lesser pipefish, sand goby, green crab, brown shrimp, and European flounder with a total water volume of 12 L. **b**: The arena we used for humbug damselfish and orange-fin anemonefish in 2024 with a water volume 8 L for fast-warming, 18 L for slow-warming; a similar arena was used in 2023 (humbug damselfish). **c**: the arena we used for brook trout, bluntnose minnow, rusty crayfish, and bluegill, with a water volume of 26 L. **d**: the arena (left = arena where the fish were confined, right = sump containing heaters, pumps, and air stones) that we used for the slow-warming sand goby and flatfish trials with a total water volume of 35 L.