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Pairing lab and field studies to predict thermal performance of wild fish

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ABSTRACT

In thermally variable ecosystems, temperatures can change extensively on hourly and seasonal timescales requiring ectotherms to possess a broad thermal tolerance (critical thermal minima [CTmin] and maxima [CTmax]). However, whether fish acclimate in the laboratory similarly as they acclimatize in the field under comparable thermal variation is unclear. We used temperature data from a tidal salt marsh to design 21-day lab-acclimation treatments (static: 12, 17, 22, 27 °C; daily variation with mean 22 °C: i) range 17-27 °C, ii) range 17-27 °C with irregular extremes within 12-32 °C). We compared thermal limits in lab-acclimated and field-acclimatized eurythermal arrow goby (*Clevelandia ios*). Variable temperature-acclimated and acclimatized fish had similar CTmin and CTmax. Notably, arrow gobies showed rapid plasticity in their absolute thermal tolerance within one tidal cycle. The daily mean and max temperatures experienced were positively related to CTmax and CTmin, respectively. This study demonstrates that ecologically informed lab acclimation treatments can yield tolerance results that are applicable to wild fish.

1. Introduction

Aquatic systems are changing at unprecedented rates due to globally rising average temperatures (+2 °C within the next 20 years; IPCC 2022) and intensifying extreme variability (Clare et al., 2022). Ectotherm' physiology, such as growth, reproduction, and digestion, depends on ambient temperatures (Fry, 1971; Schulte, 2015), and when the local temperatures become unfavorable, animals may leave, stay and acclimatize if their capacity permits, or perish (Boyd et al., 2016; Somero, 2005). In the pursuit of a better understanding of functional and absolute thermal limits in ectotherms (Bates and Morley, 2020; Desforges et al., 2023; Somero, 2005), one undesirable consequence of lab-driven studies could be a discrepancy between responses from wild-acclimatized and lab-acclimated animals (Borowiec et al., 2016; Fangue et al., 2011; Morgan et al., 2019; Rodgers et al., 2019). It is unclear whether lab-acclimated animals possess improved, similar, or impaired tolerance and performance compared to their wild counterparts. For example, in wild-caught fish compared to fish lab-acclimated to static conditions within ecologically relevant ranges, the upper thermal tolerance can be lower (Morgan et al., 2019; Fangue et al., 2011) or higher (Borowiec et al., 2016; Feldmeth et al., 1974). Possibly, some of the discrepancy between these results could be explained by the lack of natural variation in static lab-acclimations (Turko et al., 2023). Coupling lab and field observations to better predict the thermal limits of ectotherms could improve our ability to anticipate the climate responses of wild animals in vulnerable and variable habitats.

Studies on lab-acclimated animals have provided a foundational knowledge of fish thermal physiology (Schulte, 2015). In contrast to tightly controlled acclimation conditions in the lab, field acclimatized fish encompass the dynamic exposure to temperatures as well as variable predation, food availability, diet choices, social cues, refuge, oxygen levels, or salinity (Todgham and Stillman, 2013). The presence of these variables raises the question of whether fish acclimate in the lab in the same manner as they acclimatize in the field. Here, we address the challenge of designing lab studies to predict thermal limits in field-acclimatized fish. We monitored temperatures at a fine scale across three years, which allowed us to characterize the temporal and spatial heterogeneity (seasons, days, hours, and across locations) at the tidal salt marsh. Specifically, our goal was to create thermal conditions in the lab that resemble those in the field and to test critical thermal tolerance (CTmin, CTmax) in the eurythermal and abundant fish, arrow goby (*Clevelandia ios*), from a tidal salt marsh. Measuring the CTmax and CTmin in the field and the lab allowed us to compare results between acclimated and acclimatized fish.

2. Materials and methods

Animal handling and experimental protocols were approved by

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IACUC protocol 945 and collected under a CDFW permit. This study was conducted at Carpinteria salt marsh, California, USA (Fig. 1A), where temperatures were monitored at five locations between September 2019 and April 2022 using Hobo loggers (HOBO TidbiT MX Temperature 400′ Data Logger, Onset Computer Corporation, Bourne, MA, USA) (Fig. 1A). Carpinteria salt marsh is a tidal, widely heterogeneous ecosystem that serves critical roles for inland and coastal systems by providing habitat to diverse species and nursery grounds to many ectotherms, including coastal fishes (Brooks, 1994).

For the lab experiments arrow gobies (n = 595) were collected from various locations within the salt marsh and across various tidal cycle conditions using a seine net or hand nets (all collection sites were within ~300 m from the field test collection site, Fig. 1A). Fish were transported to the University of California, Santa Barbara in coolers with aerated saltwater (>90 % air saturation) where they were kept in aerated flow-through filtered seawater (25-gal tanks; 30–35 fish per tank, >85 % air saturation) under natural light cycle (14L:10D, 06:00h off 20:00h on). Fish were habituated to 17- 18 °C for 1–4 days, and then randomly assigned to one of the six temperature acclimation treatments (3 tanks/treatment; 30–35 fish/tank) (Fig. 2B). Temperatures were changed at ~ 2 °C/h rate to reach acclimation temperatures. Lab acclimations resembled seasonal and daily mean temperatures (static: 12 °C, 17 °C, 22 °C, and 27 °C) and daily temperature variability at two intensity levels: one variable treatment fluctuated consistently between 17 and 27 °C with mean 22 °C (“V1”), and one variable treatment had an additional randomly drawn temperature increase or decrease (range 12-32 °C; “V2”) every other day (Fig. 2). To achieve the target acclimation temperatures, each tank was plumbed with both warm (~21 °C) and cold (~11 °C) water, and equipped with submersible 500W heaters with a temperature control unit (INKBIRD ITC-308, INKBIRD Tech. C.L., Shenzhen, China). Two heaters were used to control the fluctuating acclimations; the first heater was turned on at 7:00h, set to heat to 22 °C and turned off at 19:00h. The second heater was turned on at 9:00h to heat from 22 °C to the target high temperature of the day, turned down to 22 °C at 17:00h and turned off at 19:00h. This approach allowed us to control for similar daily heating and cooling rates (Fig. 2B). Acclimations took place from Oct 11, 2019, until Nov 29, 2019, providing at

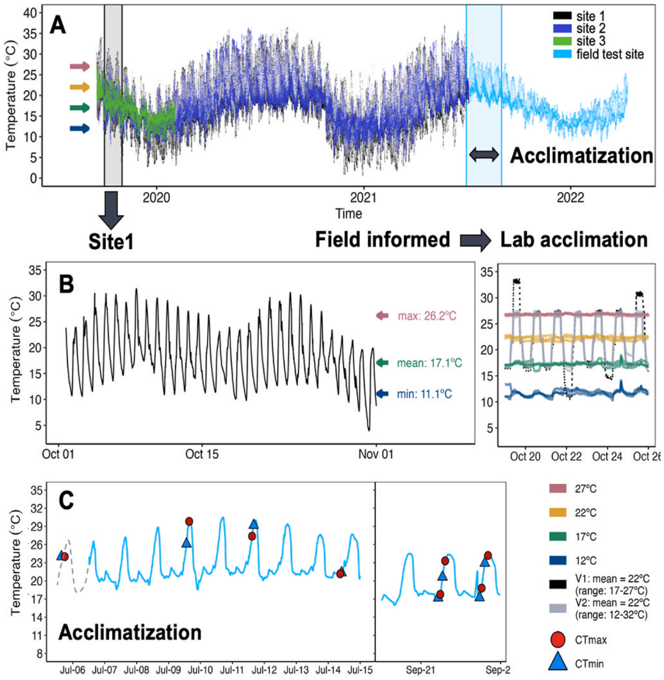
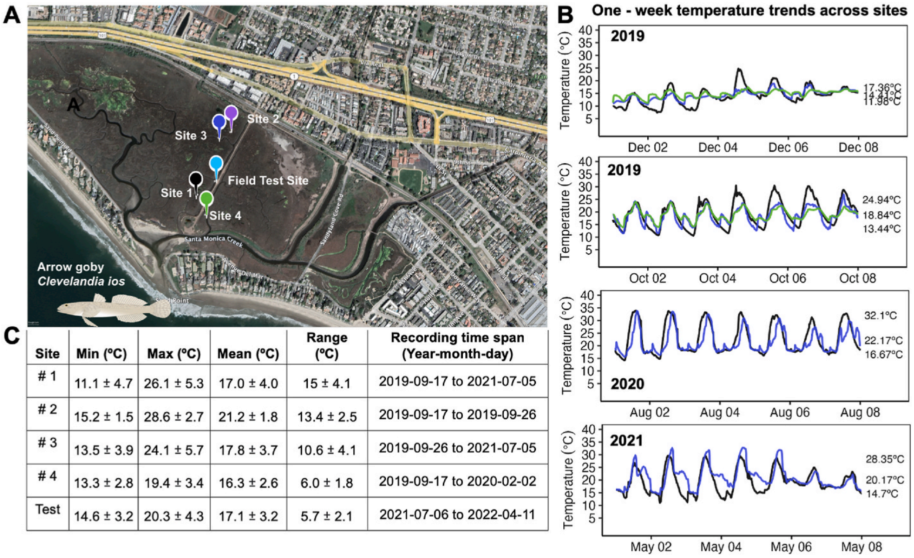


Fig. 2. Long- and short-term field temperatures were used to parameterize lab acclimation treatments. (A) Recorded temperatures in the Carpinteria Salt Marsh from July 2019 to April 2022 at different sites (colors). The arrows mark chosen environmentally relevant temperatures to design lab acclimation experiments. (**B**) On the left are the temperatures from Site-1 during fish collections for the lab study and lab acclimation period (Oct 15 - Nov 21 of 2019; the mean of daily minimum (blue), maximum (red), and mean (green) temperatures are noted on the panel), while the right shows lab acclimation temperatures, selected to mimic field temperatures (6 temperature treatments: 4 static and two variable). (**C**) Temperature during field acclimatization experiments carried out between Jul 6 and Sept 2 of 2021. The symbols mark the specific times along the environmental temperature trend when the CTmin (blue triangle) and max (red circle) tests were carried out in the field.



Site	Min (ºC)	Max (ºC)	Mean (ºC)	Range (ºC)	Recording time span (Year-month-day)
# 1	11.1 ± 4.7	26.1 ± 5.3	17.0 ± 4.0	15 ± 4.1	2019-09-17 to 2021-07-05
# 2	15.2 ± 1.5	28.6 ± 2.7	21.2 ± 1.8	13.4 ± 2.5	2019-09-17 to 2019-09-26
# 3	13.5 ± 3.9	24.1 ± 5.7	17.8 ± 3.7	10.6 ± 4.1	2019-09-26 to 2021-07-05
# 4	13.3 ± 2.8	19.4 ± 3.4	16.3 ± 2.6	6.0 ± 1.8	2019-09-17 to 2020-02-02
Test	14.6 ± 3.2	20.3 ± 4.3	17.1 ± 3.2	5.7 ± 2.1	2021-07-06 to 2022-04-11

Fig. 1. The temporal and spatial heterogeneity of tidal Carpinteria Salt marsh. Pins indicate the GPS coordinates where HOBO temperature loggers were deployed at the Carpinteria Salt Marsh between 2019 and 2022. (**A**) The map marks five sites where HOBO temperature loggers were deployed across time at varying durations (Table **C**). Arrow gobies for the field experiments were collected at the “Field Test Site” (in turquoise). (**B**) The comparison of temperature profiles at four different times and seasons (plot facets) and different sites (colors corresponding to the coordinate pins in **A**). (**C**) The summary table of measured temperatures across time and sites: the means ± s.d. of daily minimum, maximum, and average temperatures, and the range of daily temperature (maximum - minimum each day). [temperatures were downloaded bi-annually, except through 2020 due to COVID restrictions, the recording frequency was 10 min (2019–2020), 15 min (2021–2022)]. Credit for arrow goby illustration: Collin Gross.

least 21 days before testing. In the lab, water quality was measured weekly and maintained optimal (Nitrite: 0 ppm, Nitrate <10 ppm, pH: 7.8–8.4). All fish were fed once daily with commercially available fish flakes (Zoo Med's Spirulina 20, Zoo Med Laboratories Inc. San Luis Obispo, CA, USA; TetraMin, Tetra. Blacksburg, VA, USA); feeding was skipped the day before thermal tolerance tests, except for 2 CTmin trials. For the field experiments, fish (n = 275) were collected upon arrival at the site and were tested in groups of 14–23 throughout the day. The first group was tested immediately after collection, while others were transferred to a mesh bag with a plastic cap (Leclair et al., 2020) and held submerged at a collection site under natural conditions until testing.

Thermal tolerance in fish was measured according to standard CTmax and CTmin protocols (Beitinger et al., 2000). Briefly, fish were transferred to the test tank with minimal air exposure (<10 s) at their acclimation temperatures (for variable treatments, the temperature in the test tank matched the temperature in the variable treatment tank at the time, thus following the daily temperature cycle; V2 acclimations = ~22 °C and V1 acclimations = ~27 °C) (Supplemental Table 1). Fish were allowed to habituate for approximately 5 min (Firth et al., 2021; Hardison et al., 2021; Leclair et al., 2020; Turko et al., 2020), following which temperatures were changed at 0.33 °C/min rate. Throughout each test, temperatures were measured continuously using a Thermocouple thermometer (Omega, Norwalk, USA; all lab tests, 13/16 field tests) or OxyGuard handy Polaris 2 m along with measuring oxygen levels (>80 % air saturation; OxyGuard International A/S, Farum, Denmark; used for temperature during three field tests; July 9 [CTmax], July 11[CTmin and CTmax]). When an individual was unresponsive to gentle external stimuli and/or lost righting response (CTmin and CTmax), the fish was immediately removed from the test tank and placed in either a pre-labeled 100 ml beaker filled with salt water (lab experiments) or pre-labeled Tupperware with buffered 500 mg/L MS-222 to euthanize the fish (field experiments) and temperature and time were recorded. Fish were weighed (nearest 0.001 g) and measured (total length, TL, 1 mm) after the test. Field tests were performed within daylight hours (7:30h to 18:00h; 8 CTmin tests, n = 139 fish; 8 CTmax tests, n = 136). The setup for field and lab thermal tolerance tests is described in the supplemental material.

2.1. Data and statistical analysis

All data and statistical analyses were done in R version 4.2.0 (2022-04-22). The following R packages were used for data and statistical analysis data presentation: 'lme4', 'emmeans', 'stats', 'car', 'ggplot2', 'cowplot', 'chron', 'lubridate', 'zoo'. Fig. 1A was created using Google Earth Pro and the tool's 2019 aerial footage of the site. First, we statistically evaluated if CTmin and CTmax increased with increasing static acclimation temperatures using mixed models (fixed effects: temperature [continuous variable] and total length, TL [continuous variable]; random intercept effect: fish tank ID). Second, using mixed effect models, we evaluated if CTmin and CTmax were different across any of the tests (fixed effects: treatment [each lab treatment, and each field test; categorical variable] and TL [continuous variable]; random intercept effect: fish tank ID [test day representing unique collection site was assigned as a 'tank' for field tests]). Third, we used mixed models to simply compare if CTmin and CTmax differed between field and lab tests (fixed effects: location ['Lab' or 'Field', categorical variable] and TL [continuous variable]; random intercept effect: fish tank ID nested in treatment). Fourth, we explored what environmental temperature indices best explained CTmin and CTmax in acclimated and acclimatized fish using mixed models (fixed effects: i) daily mean temperatures, ii) daily max temperatures, iii) daily min temperatures, and iv) daily Δ [max - min] temperatures, v) the start temperature of the test (all in °C, continuous variable), and vi) TL (continuous variable) in various combinations; random intercept effect: fish tank ID nested in treatment). The best-fit models were those with the lowest BIC score (Supplementary Table 4), the significance of fixed factors was determined using type II ANOVA, and when appropriate, post-hoc tests with the Tukey method were used for pairwise comparisons. The residuals of all fitted models were visually assessed for normality. Significance was accepted at alpha level 0.05.

3. Results and discussion

CTmax and CTmin in arrow gobies lab-acclimated to static temperatures (12, 17, 22, 27 °C) both increased similarly and predictably with increasing acclimation temperature (CTmax: $\chi^2 = 188.87$; $p < 2e\text{-}16$; CTmin: $\chi^2 = 639.08$; $p < 2e\text{-}16$; CTmin and CTmax: size (TL) not significant; Fig. 3A). Arrow gobies were likely fully acclimated after spending ~21 days in static temperatures (Fangue et al., 2014; Pintor et al., 2016), with their mean CTmin ranging between 1.63 °C and 6.56 °C and mean CTmax between 34 °C and 39 °C. Notably, the CT window (mean CTmax - mean CTmin) was consistent at ~ 32 °C across all acclimations (Fig. 3A). In the wild, these fish rarely, if ever, experience extended static thermal conditions, thus making these results difficult to place within ecologically relevant contexts.

Some differences were discovered when all tests, field and lab, static and variable temperature acclimations, were compared. We found significant differences between field tests and lab treatments (field tests (n = 8) and lab acclimations (n = 6) included in the mixed model as a treatment) (CTmin: $\chi^2 = 379.44$, $p < 2e\text{-}16$, size (TL): $\chi^2 = 4.62$, $p = 0.032$; CTmax: $\chi^2 = 214.14$, $p < 2e\text{-}16$, size (TL): not significant). The two variable lab treatments (V1 and V2; mean 22 °C) were not distinguishable (Tukey posthoc: CTmin: t-ratio = 0.73, p = 0.99; CTmax: t-ratio: 0.37, p = 1.00) as both led to a CTmin of ~ 6 °C and CTmax ~ 38 °C. Although fish acclimated to V1 and V2 in the lab had 0.5 to 1 °C higher CTmin and CTmax compared to fish that were acclimated to static 22 °C or their common mean, this increase in CTmin and CTmax was not significant. None of the field measured CTmax tests were significantly different from the lab-acclimated static 22 °C (assumed mean temperature). Only two out of eight CTmin tests were different between field-measured fish and fish lab-acclimated to 22 °C (Tukey posthoc: Jul-9 field *vs.* 22 °C lab, t-ratio = 6.09, p = 0.0062; Jul-5 field *vs.* 22 °C lab, t-ratio = 5.16, p = 0.0166). Therefore, in this species, lab acclimation to the mean environmental temperature could generally reasonably estimate the fish's absolute thermal tolerance window. In the wild, thermal variation could serve as a biological cue allowing individuals to optimize timing within a day for foraging, digestion, metabolic recovery, or vigilance (e.g., Killen et al., 2015; Bernhardt et al., 2020) which could be enhanced at a particular temperature within a diurnal cycle (reviewed by Morash et al., 2021). Thus, more work is necessary to assess how other performance metrics (e.g., swimming, metabolism, growth, predator avoidance, feeding behavior and physiology) compare between lab and field studies (Turko et al., 2023).

The mean CT tolerance window was maintained at ~32 °C in lab-acclimated fish to either variable or static temperatures and, in most cases, field fish when measured within a day. The lowest thermal breath of 27.71 °C was measured on July 9 (daily min = 18.56, mean = 22.81, max = 29.95 °C) (CTmax = 35.9 °C ±, CTmin = 8.27 ± 1.98 °C, mean ± s.d., Supplemental Table 3, Fig. 3B), thus demonstrating that the thermal window can be flexible in arrow gobies depending on the context and time of the measurement. Acclimation and acclimatization responses where fish maintain their thermal tolerance window when exposed to different temperature regimes, including thermal cycling, have been observed in some fishes, but not all (Bennett and Beitinger, 1997; Currie et al., 2004). For example, fluctuating temperature acclimation increased the CT window in pupfish (Feldmeth et al., 1974), sheepshead minnow (Bennett and Beitinger, 1997), and Chinese seabream (Peng et al., 2014) but did not in channel catfish, rainbow trout, and largemouth bass (Currie et al., 2004). Maintenance of a broad thermal window in arrow gobies, even when acclimated to static temperatures, could be an ecologically relevant response, because they can

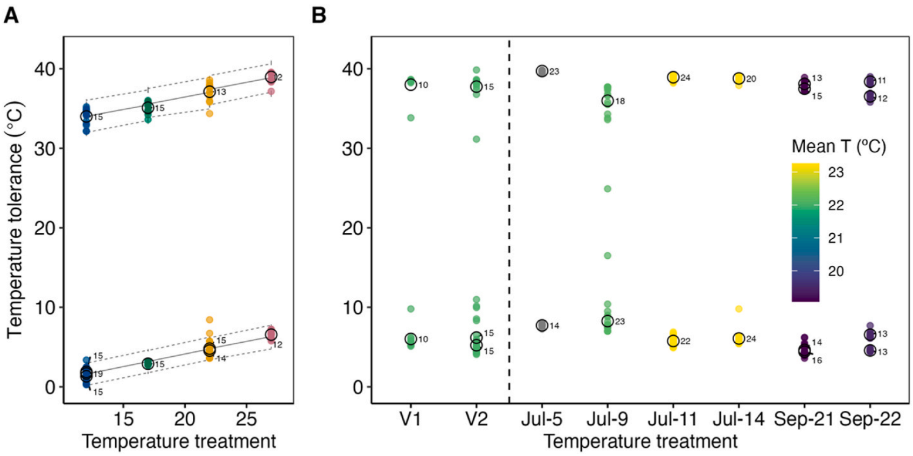


Fig. 3. CTmin (lower temperatures) and CTmax (higher temperatures) in arrow gobies acclimated for 21 days to static and variable temperatures in the lab and in arrow gobies acclimatized in the field. (**A**) Linear relationships (solid line) with 95 % confidence intervals (gray dotted line) between thermal limits of arrow gobies lab-acclimated to static temperatures. (**B**) Thermal limits varied in fish lab-acclimated to variable temperature treatments (V1 and V2; on the left side from dashed line) and field-acclimatized fish (right from dashed line). The color scale in panel B is the mean temperature of the test day. The larger empty circles in both panels are the mean CT values for each test, and the corresponding sample size is noted on the plot. The double tests on the same x-axis value are fish fed *versus* not fed for lab studies, and for repeat CT tests performed on the same day, a few hours apart, in the field.

experience acute temperature swings (either cold or warm) that are irregular and unpredictable due to variable sun exposure, depth of water, tide, and seasons. The broad and stable thermal window also could be a signature of developmental plasticity (Shaefer and Ryan, 2006), as all fish in this study were wild-caught and experienced thermal variations during development. The functional (and genetic) tradeoffs between thermal breath and plasticity in fish acclimated to static and variable temperatures are still unclear.

Lab acclimations designed to mirror the variable temperatures that ectotherms experience in the wild are becoming more popular (Morash et al., 2021; Rodgers et al., 2019; Schwieterman et al., 2022). Here, temperature tolerance in lab-acclimated fish exposed to variable treatments did not significantly differ from field-acclimatized fish (ANOVA: field vs lab variable test: CTmin: $\chi^2 = 0.64$, p = 0.42, size (TL): $\chi^2 = 11.07$, $p < 0.001$; CTmax: $\chi^2 = 0.09$, p = 0.76, size (TL): not significant; Fig. 3B). Specifically, the mean CTmin was 6.12 °C in the field fish (8 tests; n = 139) and 5.79 °C in the lab fish (V1 and V2 combined, n = 40), while the mean CTmax was 38.2 °C (8 tests, n = 136) and 37.9 °C (V1 and V2, n = 25) in field and lab fish, respectively (Fig. 3B). Tolerance limits in fish have been shown to differ in the lab versus field experiments, though the mechanistic basis remains unclear (Morgan et al., 2019; Turko et al., 2020; hypoxia tolerance in bluegill: Borowiec et al., 2016). Possibly, differences in stress and handling (e.g., netting, transfer, and test setup often occurring within a few hours of capture in the field) could impact test results. Field fish may be digesting food, which could affect thermal tolerance, though, in lab fish, we found no differences in CTmin between gobies in a fed and unfed state (12 °C, 22 °C static, and V2; Fig. 3; Supplementary Table 3; Snow, 2018). Some variability could be attributed to measurements occurring at different times of the day with different test start temperatures (e.g., here between 7:00h and 20:00h; 12.5 °C and 32 °C start temperature depending on the treatment; Supplemental Table 1; see Chown et al., 2009; Desforges et al., 2023). Lastly, field-acclimatized fish are under a natural influence of varying oxygen levels, salinity, predation, social interactions, food availability, all of which are controlled in lab-acclimated fish (Turko et al., 2023). Even still, here we found that thermal limits were indistinguishable between thermally variable lab-acclimated *vs*. field-acclimatized fish, demonstrating that carefully parametrized lab studies could reflect thermal performance of wild fish.

Absolute thermal limits can be highly flexible across short timescales, especially in eurythermal species (Desforges et al., 2023; Gunderson et al., 2016). This was particularly evident in field acclimatized arrow gobies, in which field CTmin ranged between 3.6 °C and 16.5 °C (coefficient of variation (cv%): 27.06 %; n = 139), while the CTmax range was 24.9–39.8 °C (cv: 4.37 %; n = 136). Notably, the thermal limits of the field fish differed by up to a mean of 2 °C even within the same day, tested just a few hours apart (Fig. 3B; Sept 22: post hoc test comparing CTmin: t-ratio = 4.76, p = 3e-3; CTmax: t-ratio = 3.65, p = 0.02; Sept 21 repeat tests were *ns*). This variation could be underscored by the timing (and test start temperature) of the field CTmin and CTmax tests with respect to dynamics of daily temperature cycle (Cooper et al., 2021). Additionally, CTmin was more variable among individuals (Figs. 3 and 4, Supplementary Table 3) compared to CTmax. It is clear that temperature tolerance is highly dependent on prior temperature experiences. At short timescales, the different components of daily temperature variation (e.g., mean, max, min, range) could have dominant effects on CTmin and CTmax (Somero, 2005; Cooper et al., 2021).

In arrow gobies, CTmin was best explained by the daily max temperature, the range of daily temperatures, and body length (Fig. 4B; Supplementary Table 4), while CTmax was best explained by the daily mean temperature (Fig. 4A, Fig. S1; Supplementary Table 4; non-significant size effect). Fangue et al. (2014) used static lab-acclimation treatments to show that CTmin is lost more quickly than gained, which could partly explain why daily maximum temperature was a strong predictor of CTmin in our study. Additionally, lower thermal limits were more variable and plastic compared to upper thermal limits in eurythermal cichlid (Cooper et al., 2021), and other taxonomically diverse ectotherms, including crabs and snails (Somero, 2005) and bees (Gonzalez et al., 2022) (mata-analysis: Gunderson et al., 2016). These results suggest that CTmin and CTmax are driven by different mechanisms (e.g., acclimation response (Desforges et al., 2023; Turko et al., 2023), onset of the heat shock response (Fangue et al., 2006; Pandey et al., 2021), oxidative stress and immune response (Pandey et al., 2021, Ern et al., 2023), change in membrane fluidity (Ern et al., 2023) that likely operate across different timescales (Bennett et al., 1998; Currie et al., 2004; Cooper et al., 2021; Fangue et al., 2014; Pandey et al., 2021; Pintor et al., 2016). Notably, larger arrow gobies (total length, mm) had lower CTmin but body size had no effect on their CTmax (Fig. S1). In fishes, the effects of size on absolute thermal tolerance across species is inconclusive and, in many cases, including this study, weak (negative size effect on CTmax: McPhee et al., 2023; no size effect: Turko et al., 2020; species specific effects of size on CTmin and CTmax: Di Santo and Lobel, 2017; Ospina and Mora 2004). To better understand what biological factors and thermal history factors drive CTmin and CTmax in

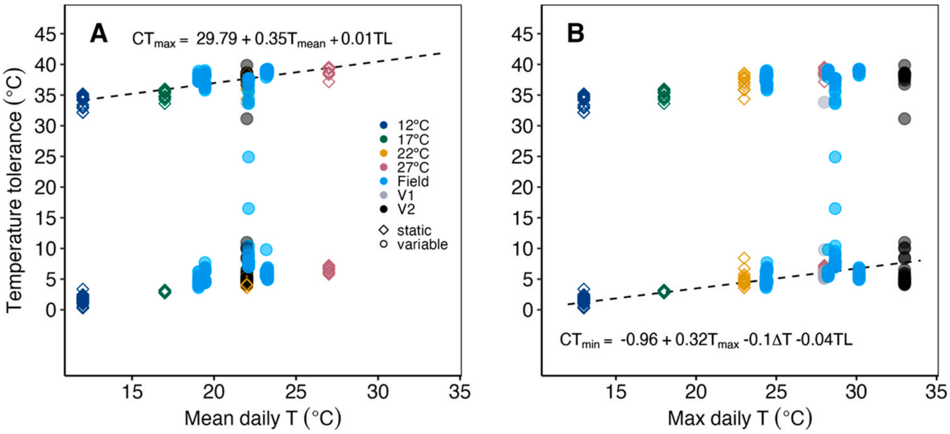


Fig. 4. Maximum and minimum thermal tolerance limits increase in arrow gobies as a function of daily environmental temperatures (mean and max). The blue symbols are field tests, the grayscale symbols are lab-acclimated variable treatment tests, and the colored diamonds are lab-acclimated static tests. Individual fish's CTmax and CTmin are plotted on each panel. Daily mean temperature (**A**) was the best temperature metric predicting CTmax (Field: $n_{tests} = 8$, $n_{individuals} = 136$; Lab: $n_{tests} = 4$, $n_{individuals} = 80$), and the daily maximum temperature and daily range (maximum – minimum temperature, Δ°C) (**B**) was the best temperature metric predicting CTmin (Field: $n_{tests} = 8$, $n_{individuals} = 139$; Lab: $n_{tests} = 7$, $n_{individuals} = 145$). TL = total length, mm. T = temperature, °C.

fishes, it will be necessary to conduct more extensive field studies, or studies that lab-acclimate fish to various types of variation (e.g., same mean with differing amplitude of temperature fluctuations, and *vice versa*).

Long term monitoring of field temperatures allows us to characterize the natural conditions of wild fish, design ecologically relevant field and lab studies, and understand what currently (and historically) may be considered optimal temperature ranges or extreme conditions. The intensity and magnitude of thermal variation varies within the tidal Carpinteria salt marsh (Fig. 1B). For example, "site 1" was a shallow tidal pool where temperatures varied most drastically all year around (daily Δ°C: 15.0 °C ± 4.06 °C, mean ± s.d., n = 658 days). In contrast, in the deeper main channels, where the residence time of tidal ocean water is longer, the daily temperature fluctuations were more moderate ("site 4" daily Δ°C: 6.03 ± 1.80, n = 139 days; "test site" daily Δ°C: 5.74 ± 2.05, n = 280 d; mean ± s.d.; Fig. 1C). It is unknown how much arrow gobies move around between different sites within the salt marsh or how much they use burrows created by other species for behavioral thermoregulation (e.g., Dawson et al., 2002). However, the ability to rapidly modulate thermal tolerance, as shown here in arrow gobies, can be beneficial for ectotherms living in such a highly variable and unpredictable habitat (Morash et al., 2021). Additionally, having continuous measurements instead of point measurements enables characterization of the rate of temperature changes at minute-to hourly timescales which can advance our understanding of how historical temperature profiles drive acute and acclimation performances of wild fish from a particular population (Eliason et al., 2011) or species (Nati et al., 2021; Sunday et al., 2012). Additionally, the accessibility of environmental data allows researchers to design ecologically relevant field and lab studies. The pairing of lab and field data (temperature exposures and thermal tolerance) provided a transferrable perspective of how static lab acclimations can overrule some of the variations in absolute thermal limits in arrow gobies, even though arrow gobies can modulate CTmin and CTmax even with one day in the field.

Future studies may expand the scope of this work by concurrently monitoring additional factors in the field (e.g., oxygen levels, added nutrient loads) and by designing multi-stressor studies in the lab with thermal variation (Desforges et al., 2023; Gunderson et al., 2016; Todgham and Stillman, 2013). Furthermore, functional thermal limit metrics, such as cardiac thermal performance (Eliason and Anttila, 2017; Schwieterman et al., 2022), swim performance (Rodgers et al., 2018), growth (Morissette et al., 2021), or behavioral assays, instead of CTmin and CTmax could provide a broader ecologically relevant evaluation of thermal physiology and habitat suitability in wild fish (Harvey et al., 2022). In summary, we showed a predictable increase (CTmax increased by 0.34 °C ± 0.03 for every degree of acclimation and by 0.32 °C ± 0.01 for CTmin [slope ± s.e.m.]) of thermal limits with increasing static acclimation temperatures, yielding a consistent CT tolerance window of 32 °C across all acclimations. The environmentally informed variable temperature acclimation treatments in the lab yielded similar tolerance patterns to field acclimatized fish, demonstrating that appropriate lab conditions can yield results applicable to wild fish. Across all treatments, the daily mean temperature was the best predictor for CTmax, and the daily maximum temperature and daily range best predicted CTmin in arrow gobies. Lastly, arrow gobies possessed a strong thermal acclimation response, and a remarkable rapid plasticity of thermal limits (up to 2 °C change across a single tidal cycle) under variable temperatures. In conclusion, we showed a case study where measuring and comparing thermal limits in lab-acclimated and field-acclimatized fish to variable temperatures could advance our understanding of climate responses of wild animals in vulnerable and variable habitats.

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CRediT authorship contribution statement

Krista Kraskura: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Claire E. Anderson:** Funding acquisition, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. **Erika J. Eliason:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

Authors declare no competing interests.

Data availability

All data and code are publicly available on the GitHub repository: https://github.com/kraskura/KK_etal_gobies_tempVar_tempTol

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2023.103780.

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