


Dynamics of thermal tolerance plasticity across fish species and life stages

Jeremy De Bonville^{a,*} , Anna H. Andreassen^b, Zara-Louise Cowan^{b,c}, Lorena Silva-Garay^b, Robine H.J. Leeuwis^b, Eirik R. Åsheim^{d,e}, Ben Speers-Roesch^f, Graham D. Raby^g, Sandra A. Binning^a, Fredrik Jutfelt^{b,h}

^a Groupe de Recherche Interuniversitaire en Limnologie et en Environnement Aquatique (GRIL), Département de Sciences Biologiques, Université de Montréal, Montréal, QC, H2V 0B3, Canada

^b Department of Biology, Faculty of Natural Sciences, Norwegian University of Science and Technology, Trondheim, 7491, Norway

^c Natural Resources Institute Finland (Luke), 90570, Oulu, Finland

^d Institute of Biotechnology, Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki, Finland

^e Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

^f Department of Biological Sciences, University of New Brunswick, Saint John, NB, E2L 4L5, Canada

^g Department of Biology, Trent University, Peterborough, ON, K9J 1Z8, Canada

^h Department of Biology and Environmental Sciences, Kristineberg Center, University of Gothenburg, Fiskebäckskil, Sweden

ARTICLE INFO

Keywords:

Acclimation response ratio
Critical thermal maximum
Critical thermal minimum
Climate change
Thermal tolerance
Plasticity

ABSTRACT

Climate warming with associated heat waves presents a concerning challenge for ectotherms such as fishes. During heatwaves, the ability to rapidly acclimate can be crucial for survival. However, surprisingly little is known about how different species and life stages vary in their acclimation dynamics, including the magnitude of change in thermal tolerance through acclimation (i.e. acclimation capacity; also known as the acclimation response ratio, ARR), the duration needed for the novel acclimation temperature to significantly alter thermal tolerance from the initial level (which we term the response induction time, $t_{\text{induction}}$), or the duration needed to achieve the new acclimation steady state (which we term the time to full acclimation, t_{steady}). To shed light on this knowledge gap, we studied the acclimation dynamics of three wild-caught fishes (goldsinny wrasse, three-spined stickleback and European flounder) by assessing upper thermal tolerance (CT_{max}) after different periods of time acclimating to a warmed environment. We also measured both CT_{max} and lower thermal tolerance (CT_{min}) in juvenile and adult lab-bred zebrafish acclimated to a warmed environment. Upper thermal tolerance of zebrafish and sticklebacks significantly increased after a 3 h exposure to a warm treatment, while $t_{\text{induction}}$ took six and 24 h in the wrasse and flounder, respectively. Goldsinny wrasse had the highest ARR, and did not reach full acclimation of CT_{max} within the duration of the study (10 days). All other species fully acclimated within 4–10 days. Juvenile zebrafish showed similar acclimation dynamics to adults for both upper and lower thermal tolerance, but had a higher CT_{min} for all acclimation durations. Our results demonstrate that acclimation dynamics of thermal tolerance vary across species, but can be similar between life stages within species. Understanding species-specific thermal plasticity is important for accurately modeling the projected impacts of climate change.

1. Introduction

Due to climate change, aquatic systems globally are facing increases in average water temperature, which poses a severe threat to ectothermic animals, such as fishes (IPCC, 2022; Jutfelt et al., 2024). Rapid warming events may pose an even greater threat than average temperature increases for many populations (Vasseur et al., 2014). Indeed,

extreme weather events are increasing in frequency and can lead to mortalities during, or in the days following, exposure to warm (Ern et al., 2023; De Bonville et al., 2024) and cold (Lubitz et al., 2024) thermal stress. Fishes can also be naturally exposed to rapid changes in temperature, for example, in shallow or dynamic waters (Vinagre et al., 2018; Morash et al., 2021; Desforages et al., 2023) or by moving through thermoclines (Amat-Trigo et al., 2023). As such, many species have

* Corresponding author. Groupe de Recherche Interuniversitaire en Limnologie et en Environnement Aquatique (GRIL), Département de Sciences Biologiques, Université de Montréal, Montréal, QC, H2V 0B3, Canada.

E-mail address: jeremy.de.bonville@umontreal.ca (J. De Bonville).

<https://doi.org/10.1016/j.jtherbio.2024.104024>

Received 24 July 2024; Received in revised form 1 October 2024; Accepted 11 November 2024

Available online 19 December 2024

0306-4565/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

evolved mechanisms allowing them to tolerate large temperature fluctuations (Leeuwis and Gampel, 2022). Since the rates of most physiological processes are affected by temperature, rapid warming or cooling will likely impact fish performance traits such as metabolic rates, swim performance, and thermal tolerance (Jutfelt et al., 2024).

Through phenotypic plasticity, ectotherms can acclimate their physiological processes to match the novel thermal conditions they are exposed to and counteract physiological stress (Angiletta, 2009; Blewett et al., 2022). Acclimation of an individual's thermal tolerance can be assessed using the critical thermal method (CTM) to evaluate the upper (CT_{max}) and lower (CT_{min}) thermal thresholds. CTM is a high-throughput method for measuring thermal tolerance and reflects the thermal acclimation of an individual as it increases or decreases with acclimation temperature (Beitinger et al., 2000; Morgan et al., 2022; De Bonville et al., 2024). Thermal acclimation dynamics, which we define as the magnitude and temporal change of thermal acclimation, has previously been studied by estimating survival time of fish transferred directly to static lethal temperatures (Loeb and Wasteneys, 1912; Doudoroff, 1942; Brett, 1970), or by estimating time-series changes in CT_{max} or CT_{min} (Chung, 1980; Bennett et al., 1998; Stewart et al., 2023). Nevertheless, there is a lack of understanding of acclimation dynamics, which could help identify which species are more at risk to the rapidly changing climate.

Acclimation dynamics can be quantified with three descriptive components. First, *acclimation capacity* refers to the magnitude of change of the initial trait value (e.g., CT_{max}) following a change in the environment (e.g., increased temperature) (Einum et al., 2019). It can be measured using the thermal tolerance gain (TT_{gain}), which represents the increase in thermal tolerance following a change in acclimation temperature (Fangue et al., 2014) and the acclimation response ratio (ARR): the slope describing the change in a trait, such as thermal tolerance, for a given change in acclimation temperature (Claussen, 1977). Typically ranging between 0 and 1, an ARR for CT_{max} of 1 would signify a perfect thermal compensation; for each 1 °C increase in the acclimation temperature, CT_{max} would increase by 1 °C, while an ARR of 0 would indicate no changes in CT_{max} following a temperature increase (Morley et al., 2019). For example, when acclimated to 5 °C above ambient temperature for an exposure time of 20 days, juvenile largemouth bass (*Micropterus salmoides*) increased their CT_{max} by 1.3 °C (TT_{gain}), for an ARR of 0.26 (Currie et al., 1998). Second, the *response induction time* ($t_{induction}$) can be measured as the duration of exposure to an environmental variable (e.g., temperature) before an observable change in the individual's trait (e.g., CT_{max}) compared to individuals at the control temperature. In a rare study reporting the $t_{induction}$ of fishes, pupfish (*Cyprinodon dearborni*) increased their upper thermal tolerance after only 3 h in a +7 °C treatment (Chung, 1980). Third, the *time to full acclimation* is the exposure duration needed to complete acclimation, that is, the time to complete physiological adjustments to a new environment and reach a new steady state for the trait (t_{steady}). For example, in the sheepshead minnow *Cyprinodon variegatus*, CT_{max} peaked and remained stable after 17 days (t_{steady}) of being exposed to a 7.5 °C increase in ambient temperature (Fangue et al., 2014). Understanding these components of acclimation dynamics is important as they can determine a species' ability to withstand extreme weather events.

Acclimation capacity varies widely among species (Beitinger et al., 2000), largely due to adaptive or phylogenetic differences (O'Dea et al., 2022). For example, ARR is greater in channel catfish (*Ictalurus punctatus*) compared with largemouth bass of similar size and collected at the same site (Currie et al., 1998). Differences between species can also be predicted for $t_{induction}$, based on the magnitude and rapidity of thermal change typical of their environment. The few studies on fish thermal acclimation dynamics (i.e. incorporating a time course experimental design) usually do not evaluate $t_{induction}$ in the first few hours following a transfer to a warm treatment. For instance, the effects of acclimation on CT_{max} were only tested after 24 h in brook trout (Stewart et al., 2023) and common killifish (*Fundulus heteroclitus*) (Healy and Schulte, 2012),

after two days in diamond killifish (*Adinia xenica*) (McAcy, 2015) and after one week in qingbo (*Spinibarbus sinensis*) (Fu et al., 2018). Furthermore, although a southern subspecies of killifish (*Fundulus heteroclitus heteroclitus*) had a higher CT_{max} than their northern counterpart (*Fundulus heteroclitus macrolepidodus*), they both showed a significant increase in their CT_{max} after 24 h in a warm +10 °C treatment (Healy and Schulte, 2012). In all these cases, it is possible that an earlier response occurred, as observed in channel catfish which showed an increase in CT_{max} as rapidly as 7 h after being transferred from 20 to 30 °C (Bennett et al., 1998). A longer $t_{induction}$ could be expected for fish whose environment allows them to behaviourally thermoregulate (Abram et al., 2017), while it should be shorter if they are mostly found in a shallow environment with a high thermal homogeneity (Einum and Burton, 2023). Although certain fishes could have a fast $t_{induction}$ in response to changes in temperature, there is little information on the gain in thermal tolerance in the hours following the onset of acclimation. Investigations into the time to full acclimation (t_{steady}) of thermal tolerance suggest that this trait varies widely among species. Following a change in temperature, CT_{max} can stabilize between one to three days for killifish (Healy and Schulte, 2012), in three days for pupfish (Chung, 1980), but in up to 17 days for sheepshead minnows (*Cyprinodon variegatus*) (Fangue et al., 2014), 20 days for channel catfish (Bennett et al., 1998), and between 16 and 30 days for brook trout (Stewart et al., 2023). Differences in t_{steady} may be due to different acclimation strategies, even for closely related species: t_{steady} ranges from three to 17 days for two *Cyprinodon* species (Chung, 1980; Fangue et al., 2014). Different subspecies of killifish can even show a different t_{steady} based on their environment: the CT_{max} of the southern subspecies stabilized after only one day, while it took three days for the northern subspecies (Healy and Schulte, 2012). Although work on acclimation dynamics has been done, the metrics and methods used can vary among species and most studies are focused on only one species. Interspecific differences in acclimation dynamics could be key to explain differences in geographic distributions and vulnerability to heat waves.

Different acclimation dynamics among life stages could additionally be expected due to differences in size or declining plasticity with age (Dufty et al., 2002; Fischer et al., 2014). Rohr et al. (2018) suggested that t_{steady} should scale negatively with body size in ectotherms, but that acclimation capacity increases with size. This means that larger organisms acclimate slower, but to a larger extent than smaller ones. Within species, differences in thermoregulatory behaviour can vary with body size and between life stages (Wilbur et al., 2020; Amat-Trigo et al., 2023), which could give rise to stage-specific divergences in acclimation mechanisms and dynamics. Yet, there is limited research on the acclimation dynamics across life stages of a species (Burton et al., 2020; Ruthsatz et al., 2024). In contrast to Rohr et al. (2018) who only used two measurement time points, Einum and Burton (2023) argued that the acclimation rate (comparable to t_{steady}) decreases with body size. Meanwhile, no differences were found between thermal tolerance and acclimation capacity of juvenile and adult delta smelt (*Hypomesus transpacificus*) (Komoroske et al., 2014).

Thus, data on the various aspects of acclimation dynamics remain scarce (Einum and Burton, 2023). Here, we measured the acclimation dynamics of thermal tolerance in four teleosts (goldsinny wrasse [*Ctenolabrus rupestris*], three-spined stickleback [*Gasterosteus aculeatus*], European flounder [*Platichthys flesus*] and zebrafish [*Danio rerio*]; hereafter "wrasse", "stickleback", "flounder" and "zebrafish"), including two life stages for the zebrafish: juveniles and adults. We further assessed if the effects of thermal acclimation were stable through time by exposing juvenile zebrafish to an extended acclimation duration of 275 d. We predicted that (1) CT_{max} will increase with acclimation duration until it reaches a steady state and that the time course of these changes will be species-specific, that (2) mass will influence acclimation dynamics and that (3) there will be differences in acclimation dynamics between life stages for both CT_{max} and CT_{min} due to their size difference and the effect of development. We further discuss differences in

acclimation dynamics (acclimation capacity, $t_{\text{induction}}$ and t_{steady}) among species based on their distinct ecology.

2. Methods

Experiments were conducted at the Kristineberg Centre for Marine Research and Innovation (58.24965 N, 11.44585 E), University of Gothenburg, by the Gullmar fjord, Sweden (Experiment 1) and at the Norwegian University of Science and Technology (NTNU) in Trondheim, Norway (Experiment 2) from May to June 2022, with trials for a long acclimation treatment completed in February 2023.

2.1. Experiment 1: acclimation dynamics of wild marine fishes

2.1.1. Fish collection and husbandry

We used three wild-caught fish species (wrasse, stickleback and flounder). A beach seine (1 × 8 m, 3 mm mesh) was used to collect stickleback and flounder in shallow (0–2 m depth) sandy areas close to the Kristineberg research station from June 11–17, 2022. Baited lobster traps were set for approximately 1 h to collect wrasse in rocky areas of the fjord (1–3 m depth) from May 17 to June 16, 2022. After collection, sticklebacks and flounders were transported to the lab and kept in holding tanks (60 × 38 × 36 cm [L × W × H], 30 L), which received filtered flow-through water with physicochemical conditions similar to collection sites (means ± SDs: temperature: 14.78 ± 0.24 °C; salinity: 26.1 ± 0.5 ‰, data collected from the research station monitoring system, surface temperature, June 11–12, 2022 at <http://www.weather.loven.gu.se/kristineberg/en/http://www.weather.loven.gu.se/kristineberg/en/data.shtml>). The ambient water temperature was increased and kept at 18 °C over 2 days, starting June 13 (mean ± SD: 18.08 ± 0.11 °C, data collected by 2 RBRsolo³ temperature loggers [RBR Ltd, Canada] deployed in tanks on the same flow-through system). Following collection, wrasse were brought into the lab and held in larger tanks with black goby (*Gobius niger*) (80 × 75 × 51 [L × W × H] cm, 306 L), or with black goby and corkwing wrasse (*Symphodus melops*) (275 × 79 × 62 [L × W × H] cm, 1350 L), neither of which were used in this study. Conditions followed warming spring conditions (temperature: 13.11 ± 1.46 °C; salinity: 27.5 ± 1.5 PSU, from May 17 to June 15, 2022) but stabilized in the 10 days prior to the start of experiments (temperature: 14.74 ± 0.32 °C; salinity: 27.0 ± 0.8 PSU, measured from June 5–15, 2022). Flounder were fed daily to satiation with thawed commercial fish food (mysid shrimps, Akvarie Teknik). Stickleback and wrasse were fed *Euphasia* shrimp, *Pandalus* shrimp, blue mussels and Alaskan pollock, as well as wild-caught shrimps (*Crangon* sp.) for wrasses. All tanks were supplemented with plastic algae and plastic pipes for shelter and air stones for oxygenation. To mimic natural conditions, photoperiod was set to 18/6 day/night cycle.

2.1.2. Acclimation treatments

Our protocol to study the thermal acclimation response builds upon that of Bennett et al. (1998). Fish were transferred from holding tanks to experimental tanks to start acclimation to either a control or warm treatment (60 × 38 × 36 cm [L × W × H], 30 L; approximately 10 fish per species in each experimental tank [flounder: 9–10, wrasse: 6–10, stickleback: 9–11]). Control temperatures during the experiment were set to the ambient temperature at the site where fish were captured: 16 °C for wrasse caught in deeper water and 18 °C for stickleback and flounder caught in a shallow area (actual temperatures during holding: means ± SDs: 16.29 ± 0.55 °C and 18.03 ± 0.09 °C respectively). Fish were kept in experimental tanks at control temperatures for acclimation durations of 3 h–10 d (the shortest and longest durations of the warm treatment). This was done to account for the effect of handling (3 h duration) and for any lab acclimation effect (10 d duration). Upper thermal tolerance trials (CT_{max}) were done after each duration. The acclimation duration of 3 h to control temperatures is hereafter referred to as the 0 h duration. We applied a +5 °C change for the warm

treatment: 21 °C for wrasse, 23 °C for stickleback and flounder (actual temperatures during holding: means ± SDs: 20.94 ± 0.17 °C and 22.74 ± 0.22 °C respectively). Fish were directly transferred from holding tanks at control temperatures to the experimental warm tanks and tested for thermal tolerance in the warm treatment after 3 h, 6 h, 24 h, 4 d or 10 d (Fig. 1) via CT_{max} trials. No mortality was recorded during the acclimation period.

2.2. Experiment 2: acclimation dynamics of lab-bred zebrafish

2.2.1. Fish husbandry

We used adult (8th generation, ~1 year old, n = 170) and juvenile (9th generation, ~2 months old, n = 270) zebrafish, whose ancestors were collected in North Bengal, India in 2016 and lab-bred by random mating (Sundin et al., 2019; Morgan et al., 2020), for Experiment 2. Prior to the experiments, fish had been kept in captivity at 28 °C for multiple generations under a light cycle of 12/12 day/night and fed once per day with commercial fish pellets (TetraPRO Energy Multi-Crisp) for adults, and artemia and ground pellets for juveniles.

2.2.2. Acclimation treatments

Zebrafish were transferred to well-aerated tanks (59.5 × 35 × 35 cm [L × W × H], 63 L), containing an air-lift sponge filtration system and artificial plants, which were maintained for 21 d at 34 °C (warm treatment, actual temperatures: means ± SDs: 34.00 ± 0.51 °C) or 28 °C (control treatment, actual temperatures: means ± SDs: 27.78 ± 0.30 °C). Temperatures remained stable in tanks where juveniles were acclimated to 34 °C for 275 d (means ± SDs: 33.91 ± 0.54 °C). A maximum of 60 adult or 90 juvenile fish (maximum densities of 0.95 and 1.43 fish L⁻¹, respectively) were added to each tank (2 or 3 tanks per duration, for adults and juveniles, respectively). Tank temperatures were controlled by a thermostat (TC-306T, Inkbird, China) and logged every 2 min using Picologgers (Picotech TC-08, UK) or RBRsolo³ loggers. All fish were maintained on the same diet and light regime as prior to their transfer to the acclimation tanks. Lower (CT_{min}) and upper (CT_{max}) thermal tolerance were measured in groups of fish after acclimation durations of 3 h, 6 h, 24 h, 4 d, 10 d, 21 d (adults and juveniles) and 275 d (juveniles only) (Fig. 1). No mortality was recorded during the acclimation period.

2.3. Thermal tolerance trials

We used the same standardized method for all species, described by Morgan et al. (2018). Briefly, fasted fishes (between 20 and 32 h) were transferred to a testing arena at their acclimation temperature, where they were left to habituate for 5 min before heating or cooling began. Water temperature increased or decreased at a rate of 0.3 °C min⁻¹ (CT_{max} : 0.32 ± 0.04 °C min⁻¹, CT_{min} : 0.30 ± 0.02 °C min⁻¹ [means ± SDs]) using heating coils or a chiller, as well as water pumps to homogenize the water temperature throughout the arena. Water temperature and ramping rates were monitored throughout trials using a digital thermometer (testo-112, Testo, Lenzkirch, Germany, accuracy: ±0.2 °C, resolution: 0.1 °C). All species, except wrasse, were tested inside the testing arena described by Morgan et al. (2018). The setup consisted of a tank (25 × 20 × 18 cm [L × W × H], 12 L) with a mesh dividing the fish compartment from the heating compartment, which contained a 300 W coil heater and a water pump (Eheim Universal 300, Germany) for mixing. For CT_{min} , the same setup was used and connected to a cooler (Titan 500, AquaMedic, Germany). Wrasse were tested in a larger Styrofoam arena (50 × 32 × 32 cm [L × W × H], 17.5 cm water depth), which was connected to a Styrofoam heating sump (37 × 37 × 34.5 cm [L × W × H], 6.5 cm water depth) (combined volume of 35 L). Water in the sump was heated using two titanium heating rods (500 W and 300 W, Aqua Medic, Germany) and transferred to the arena using three water pumps (Eheim Universal 300, Germany): two pumps transferred water from the sump to the arena, while another pump drew water from

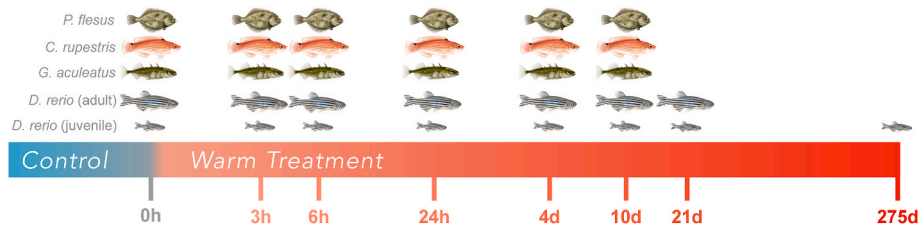


Fig. 1. Experimental design showing the four fish species and two life stages of zebrafish used in the study on a logged time scale representing acclimation duration. Fishes were initially held at a control temperature representing ambient conditions during capture in the wild (16 °C: wrasse; 18 °C: stickleback and flounder) or rearing temperatures in the lab (28 °C: zebrafish) and then were transferred to a warm treatment of either +5 °C (wrasse, stickleback and flounder) or +6 °C (zebrafish). At each time point, CT_{max} was assessed for all species, and CT_{max} as well as CT_{min} were assessed for both life stages of zebrafish.

the arena to the sump through an outlet in the bottom (sealed by mesh).

CT_{max} and CT_{min} were defined as the temperature inducing loss of equilibrium (LOE, i.e. fish could not hold an upright body position) for >3 s (Beitinger et al., 2000; Morgan et al., 2019). Upon reaching this experimental endpoint, the individual was transferred to an isolated numbered tank at their acclimation temperature, monitored for recovery and survival for 30 min, and then euthanized in 0.2 g L⁻¹ MS-222, before being weighed and measured. For logistical reasons, the number of trials for each acclimation duration varied between species and life stages: three CT_{max} trials were done for juvenile zebrafish (5 fish per trial, n = 135 for the entire time course), two for adult zebrafish (5 fish per trial, n = 80) and one for the wild-caught species (stickleback [9–11 fish per trial, n = 69], flounder [9–10 fish per trial, n = 69] and wrasse [6–10 fish per trial per trial, n = 61]). For each acclimation duration, three CT_{min} trials were done for juvenile zebrafish (5 fish per trial, n = 135) and two for adult zebrafish (5 fish per trial, n = 80). Two juvenile zebrafish were excluded from the CT_{max} trials as they escaped the arena during the trial and one stickleback as it was lethargic during the trial.

2.4. Statistical analyses

Data analyses and visualizations were conducted in RStudio, version 2022.12.0 + 353 (RStudio R Core Team, 2022) with R, version 4.2.2 (R Core Team, 2022). For all types of models, we used $p < 0.05$ as the level of statistical significance.

2.4.1. Experiment 1: Linear models

We used linear models (lm function) to assess the effect of acclimation duration (0h, 3h, 6h, 24h, 4 days, 10 days), fish mass and their interaction on the thermal tolerance of species. Separate linear models were conducted for each species. To compare the thermal tolerance across the different durations of acclimation, acclimation duration was treated as a categorical explanatory variable (Healy and Schulte, 2012; Fu et al., 2018; Stewart et al., 2023). We conducted post-hoc contrast tests of least square means with the emmeans package (Lenth, 2023) to compare differences between acclimation durations, allowing us to estimate the response induction time ($t_{induction}$) and the time to full acclimation (t_{steady}). If the interaction between acclimation duration and time was not significant ($p > 0.05$), they were not included in post-hoc comparisons to focus on the main effect of acclimation duration (Tarapacki et al., 2021).

2.4.2. Experiment 2: Linear mixed effects models

We used linear mixed effects models (lmer function) to assess the interactive effects of acclimation duration (0h, 3h, 6h, 24h, 4 days, 10 days, 21 days), life stage and fish mass on the thermal tolerance of zebrafish (one model for CT_{max} and another for CT_{min}). The packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) were used for the linear mixed effects models and to evaluate statistical significance (p -values) with Satterthwaite’s degrees of freedom method. Mixed effects models included “Tank” as a random effect as there were multiple tanks used for each acclimation duration for zebrafish (2 for

adults and 3 for juveniles), as well as interactions between acclimation duration and life stage, and life stage and mass. Although we presented it graphically, the acclimation duration of 275 d was excluded from the model, as only juveniles were tested and they had grown to an adult size (Fig. S2). Models were tested against fixed-effects-only models to validate the contribution of the random effect of “Tank”. We estimated $t_{induction}$ and t_{steady} following post-hoc contrasts tests of least square means. Non-significant interactions ($p > 0.05$) were not included in the post-hoc analysis to facilitate interpretation (Tarapacki et al., 2021).

2.4.3. Comparing species acclimation capacity

To estimate acclimation capacity and compare how species adjusted their thermal tolerance through time, we evaluated for each species the thermal tolerance gain (TT_{gain}) at each duration (t) using the following equation:

$$TT_{gain} : Average CT_{max_t} - Average CT_{max_{control}} \quad [1]$$

Where t represents the duration at which CT_{max} is measured and $CT_{max_{control}}$ is the average CT_{max} at the control temperature, prior to the onset of acclimation (“0 h”).

To compare the TT_{gain} between species of both experiments, we used a linear model to test the interaction of acclimation duration and species on the thermal tolerance gained. For this model, adult and juvenile zebrafish were considered as different “species”. We then calculated the acclimation response ratio (ARR) using the equation proposed by Clausen (1977):

$$ARR = \frac{CT_{max[T_2]} - CT_{max[T_1]}}{T_2 - T_1} \quad [2]$$

where T represents the temperature (T_2 = warm treatment [21, 23 or 34 °C] and T_1 = control temperature [16, 18 or 28 °C]). $CT_{max[T_2]}$ represents average CT_{max} at the duration where it was the highest for a given species and $CT_{max[T_1]}$ is the average CT_{max} at the control temperature, prior to the onset of acclimation (“0 h”).

Assumptions of homoscedasticity and normality of residuals were verified visually using diagnostic plots and met for all models. Using either independent samples t-tests or Mann-Whitney U tests based on data assumptions, we compared thermal tolerance at control temperature between 3 h and 10 d for the first experiment, and 3 h and 21 d for the second experiment. For visual purposes and analyses, we renamed the groups which spent 3 h at control temperature as “0 h”.

3. Results

3.1. Upper thermal tolerance across species

As expected, CT_{max} increased after 10 d of acclimation to the +5 °C treatment compared to the control temperature (0 h) for flounder (30.53 ± 0.20 °C to 31.77 ± 0.12 °C [mean ± S.E.]), wrasse (28.92 ± 0.08 °C to 31.74 ± 0.16 °C [mean ± S.E.]) and stickleback (32.84 ± 0.09 °C to 34.23 ± 0.07 °C [mean ± S.E.]) (Table S1). Between species, stickleback

had the highest thermal tolerance at 0 h, even compared to 10 d + 5 °C acclimated groups of the other two species (Fig. 2). However, the wrasse had the highest acclimation capacity, with a TT_{gain} of 2.82 °C (ARR = 0.56) at the end of the 10 d acclimation duration, while flounder and stickleback had a TT_{gain} of 1.24 °C (ARR = 0.25) and 1.39 °C (ARR = 0.28), respectively. For wrasse and stickleback, there was a significant increase in CT_{max} at control temperatures (16 °C and 18 °C, respectively) between 3 h and 10 d, but none for flounder at 18 °C (Table S3).

All three species increased their CT_{max} in response to warm acclimation, however their temporal patterns of CT_{max} differed en route to their day 10 ‘final’ CT_{max} values (Fig. 2). For flounder CT_{max} , we found a significant interaction between acclimation duration and mass (Table 1 and Fig. S1). CT_{max} in flounder did not significantly differ between 0, 3, 6, and 24 h of acclimation, but was increased at 4 d, with no further increase in CT_{max} from 4 to 10 d ($p < 0.01$; Fig. 2). For wrasse, CT_{max} increased significantly with acclimation duration (Table 1 and Fig. 2), but there was no individual effect of mass or interaction between mass and duration. Unlike the flounder, the CT_{max} of wrasse appeared to continually increase throughout the 10 d experiment, including statistically significant increases from 0 to 6 h, 6–24 h, and from 4 to 10 d ($p < 0.05$; Fig. 2). Similar patterns were found in stickleback, where CT_{max} significantly increased with acclimation duration (Table 1 and Fig. 2), but not with fish mass or their interaction. Stickleback upper thermal tolerance increased by 0.89 °C in the first 3 h compared to the ambient treatment, but then showed a small and gradual increase throughout the remainder of the experiment, peaking after 4–10 days ($p < 0.01$; Fig. 2). See supplementary material (Table S4) for detailed model outputs.

3.2. Thermal tolerance across life stages

There was an increase in CT_{max} between ambient (0 h) and 21 d of acclimation to a +6 °C treatment for both adult (40.97 ± 0.07 °C to 43.36 ± 0.13 °C [mean ± S.E.]) and juvenile (41.08 ± 0.09 °C to 43.17 ± 0.08 °C [mean ± S.E.]) zebrafish (Table S2), with no further increase in CT_{max} from 21 to 275 d for juveniles (43.32 ± 0.15 °C [mean ± S.E.]). After 21 d, acclimation capacity was similar between life stages: TT_{gain} was 2.39 °C for adults and 2.09 °C for juveniles (relative to ambient) and their ARR was 0.4 and 0.37, respectively. The t_{steady} was comparably short across life stages as no further increases in CT_{max} occurred after 4 d (Fig. 3). For both life stages, $t_{induction}$ was 3 h, shown by the increase in CT_{max} between 0 and 3 h ($p < 0.05$; Fig. 3). There was no significant difference in CT_{max} at the ambient temperature (28 °C) between 3 h and 21 d, regardless of the life stage (Table S3).

Table 1

Analysis of variance table for the three linear models of species from Experiment 1. Significant predictors and their p -value are shown in bold.

Response	Predictors	d.f.	F-value	p -value	R^2_{aj}
Flounder CT_{max} (n = 59)	Duration	5, 47	16.40	2.405⁻⁹	0.711
	Mass	1, 47	14.00	4.975⁻⁴	
	Duration * Mass	5, 47	2.50	0.0433	
Wrasse CT_{max} (n = 51)	Duration	5, 39	56.20	<2⁻¹⁶	0.857
	Mass	1, 39	0.44	0.513	
	Duration * Mass	5, 39	0.42	0.831	
Stickleback CT_{max} (n = 59)	Duration	5, 47	34.31	1.35⁻¹⁴	0.747
	Mass	1, 47	1.01	0.321	
	Duration * Mass	5, 47	0.65	0.662	

The increase in CT_{max} with acclimation duration followed a similar pattern for both life stages, as shown by the lack of an overall difference between life stages and the lack of a significant interaction between duration and life stage (Table 2). Mass did not have an overall effect on CT_{max} within each life stage or across acclimation duration (Table 2, Fig. S2). See supplementary material (Table S5) for detailed model outputs.

CT_{min} increased for both life stages across the warm acclimation duration, indicating a loss of cold tolerance over time. From 0 h (ambient) to 21 d of acclimation, CT_{min} increased for adults (9.85 ± 0.11 °C to 12.74 ± 0.13 °C [mean ± S.E.]) and juveniles (10.21 ± 0.09 °C to 14.08 ± 0.15 °C [mean ± S.E.]) (Table S2). Interestingly, adults showed a better acclimation capacity to cold, as they only lost 2.89 °C of cold tolerance from 0 h to 21 d (from 9.85 ± 0.11 °C to 12.74 ± 0.13 °C [mean ± S.E.]), while juveniles lost 3.87 °C (from 10.21 ± 0.09 °C to 14.08 ± 0.15 °C [mean ± S.E.]). This is reflected in their ARR, which was 0.48 for adults and 0.65 for juveniles, representing the loss of lower thermal tolerance for each increase of 1 °C in the acclimation temperature. However, rates of acclimation were similar between both stages as CT_{min} followed similar patterns: peaking by 10 d with no

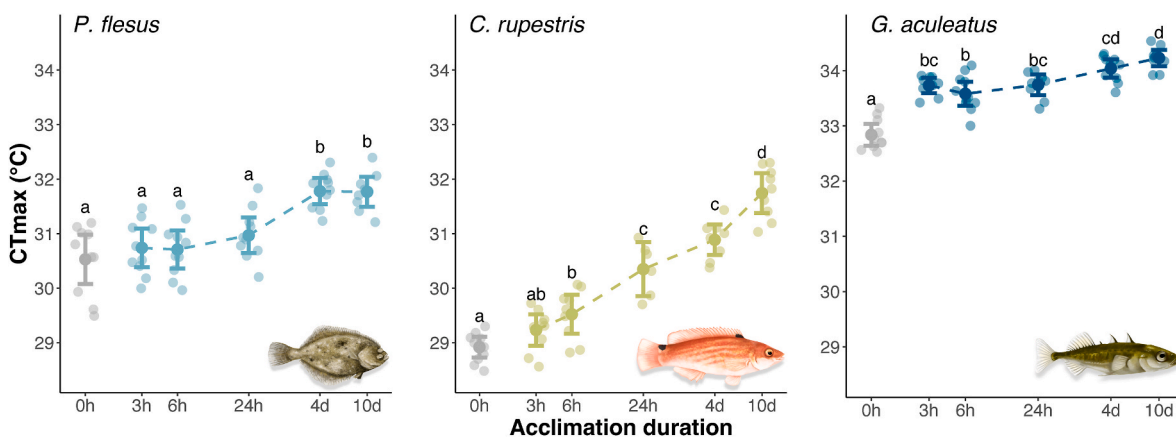


Fig. 2. CT_{max} of three marine species (European flounder *Platichthys flesus*, goldsinny wrasse *Ctenolabrus rupestris* and three-spined stickleback *Gasterosteus aculeatus*) during a 10 day acclimation to a warm (+5 °C) treatment. Individuals are represented by small transparent points. Larger dark points represent the mean for each group and error bars indicate the 95% confidence intervals. Trials conducted at 0 h (in light grey) represent thermal tolerance at the control holding temperature (wrasse: 16 °C, flounders and sticklebacks: 18 °C), for comparison against the warm treatment (wrasse: 21 °C, flounders and sticklebacks: 23 °C). Although acclimation duration is used as a categorical factor, it is displayed on a logarithmic scale to help visualize differences in time among groups. For each species, letters represent significant differences between the means of each group (Tukey’s post-hoc test, $p < 0.05$).

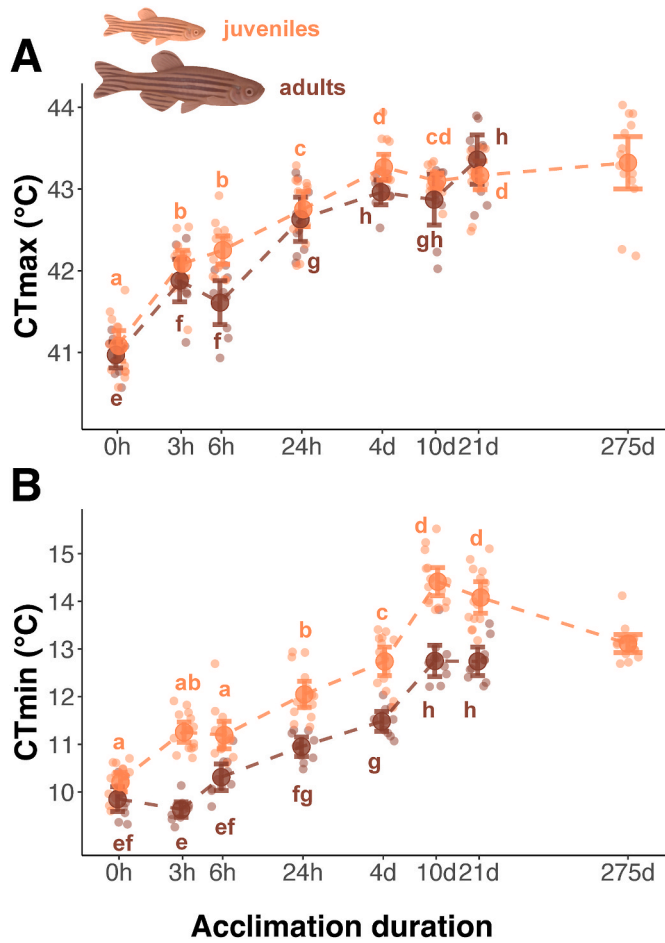


Fig. 3. CT_{max} (A) and CT_{min} (B) of adult and juvenile zebrafish (*Danio rerio*) during acclimation to a warm (+6 °C; 28 °C at 0 h to 34 °C for subsequent durations) treatment. Individuals are represented by small transparent points. Larger dark points represent the mean for each group and error bars indicate the 95% confidence intervals. Acclimation duration is displayed on a logarithmic scale to help visualize differences in time among categorical groups. For each life stage, letters represent significant differences between the means of each group (Tukey's post-hoc test, $p < 0.05$; a–d for juveniles, e–h for adults).

further change at 21 d (Tukey's post-hoc test, $p < 0.05$; Fig. 3). Interestingly, after aging juveniles to 275 d under warm acclimation conditions, they gained back some cold tolerance, with CT_{min} dropping by 0.97 °C compared to the 21 d-acclimated fish.

The $t_{induction}$ was longer for CT_{min} than for CT_{max} : no difference was observed in CT_{min} until 24 h for both life stages, compared with a 3 h response time for CT_{max} (see above). The t_{steady} was also slower for CT_{min} than CT_{max} , being of 10 d and 4 d respectively. Although there was no significant difference in CT_{min} between 3 h and 21 d at ambient temperature for adults, juveniles did show a significant increase (Table S3), between 3 d (10.21 ± 0.09 °C [mean \pm S.E.]) and 21 d (10.51 ± 0.07 °C [mean \pm S.E.]).

Similar to the patterns we observed in CT_{max} , there was no interaction between duration of acclimation and life stage and between mass and acclimation duration; however, CT_{min} increased with acclimation duration (Table 2). We also found a significant interaction between life stage and mass (Table 2), where CT_{min} was lower in heavier juveniles, but not adults (Fig. S2). See supplementary material (Table S6) for detailed model outputs.

3.3. Thermal tolerance gain differs among species

As there were no differences in CT_{max} for zebrafish among 4, 10 and

Table 2

Analysis of variance table with Satterthwaite's method for the life stages mixed effects model for both CT_{max} and CT_{min} models for Experiment 2. Significant predictors and their p -value are shown in bold.

Response	Predictors	d. f.	Den d. f.	F-value	p-value	R_m^2	R_c^2
CT_{max} (n = 174)	Duration	6	55.89	12.18	$1.04 \cdot 10^{-8}$	0.833	0.872
	Life stage	1	62.75	0.22	0.638		
	Mass	1	144.62	1.24	0.267		
	Duration *	6	45.82	0.87	0.528		
	Life stage						
	Life stage *	1	144.99	3.24	0.0740		
	Mass						
CT_{min} (n = 175)	Duration	6	61.73	29.52	$< 2 \cdot 10^{-16}$	0.918	0.953
	Life stage	1	67.23	38.70	$3.61 \cdot 10^{-8}$		
	Mass	1	151.70	69.88	$3.75 \cdot 10^{-14}$		
	Duration *	6	45.10	0.65	0.691		
	Life stage						
	Life stage *	1	147.96	45.65	$3.03 \cdot 10^{-10}$		
	Mass						
	Duration *	6	115.30	0.64	0.701		
	Mass						

R_m^2 represents the marginal R^2 while R_c^2 represents the conditional R^2 .

21 d (Fig. 3), we only included TT_{gain} up to the 10th day of acclimation for zebrafish in the linear model. This allowed us to compute the interactions between species and durations and directly compare with the other species, which were tested only up to 10 d. However, zebrafish CT_{max} at 21 days was still displayed with other acclimation durations for visual purposes and to calculate ARR (Fig. 4). The linear model examining the effects of duration, species, and their interaction on the TT_{gain} revealed a significant effect of the interaction (LM: Duration \times Species; $F_{16,239} = 8.16$, $p < 0.001$; Table S7). When comparing with flounder as the intercept, the TT_{gain} for wrasse was not significantly different at 3 and 6 h, but differed for longer duration (24 h, 4 d, 10 d; Fig. 4, Table S10). For stickleback, TT_{gain} differed as early as 3 h, but not for following durations, reflected by their rapid response induction time. Zebrafish TT_{gain} was different from flounder for all durations, except for adults acclimated for 6 h. After 4 d, flounder and juvenile zebrafish seemed to have reached a plateau in their acclimation capacity (i.e. 4 d was the time to full acclimation), as the highest average CT_{max} values for both species were recorded after 4 d and did not further increase at 10 or 21 d. For wrasse and stickleback, maximum thermal tolerance was reached only after 10 d (Fig. 4). Adult zebrafish TT_{gain} was maximized after 21 d in the warm treatment, but it was not significantly different than at 4 or 10 d. See supplementary material (Tables S7 and S8) for detailed model outputs.

For our specific temperatures, ARR was highest for wrasse (0.56), followed by adult (0.40) and juvenile (0.37) zebrafish, and lowest for stickleback (0.28) and flounder (0.25) (Fig. 4). We found that using acclimation duration as a continuous variable did not qualitatively change results and modify significant effects for all models presented (Tables S9, S10, S11).

4. Discussion

Our study provides evidence that thermal acclimation dynamics can differ considerably across fish species, but not between adult and juvenile life stages in zebrafish. Notably, our study contributes to the developing interest in acclimation dynamics, providing a substantial advancement in the number of species on which dynamics have been investigated. We also present the first CT_{max} measurements to date for the goldsinny wrasse and European flounder, both common coastal fishes of the Northeast Atlantic Ocean. Our findings underscore the critical importance of considering the temporal scale of acclimation in studies on thermal plasticity and the strategies used by fishes to cope

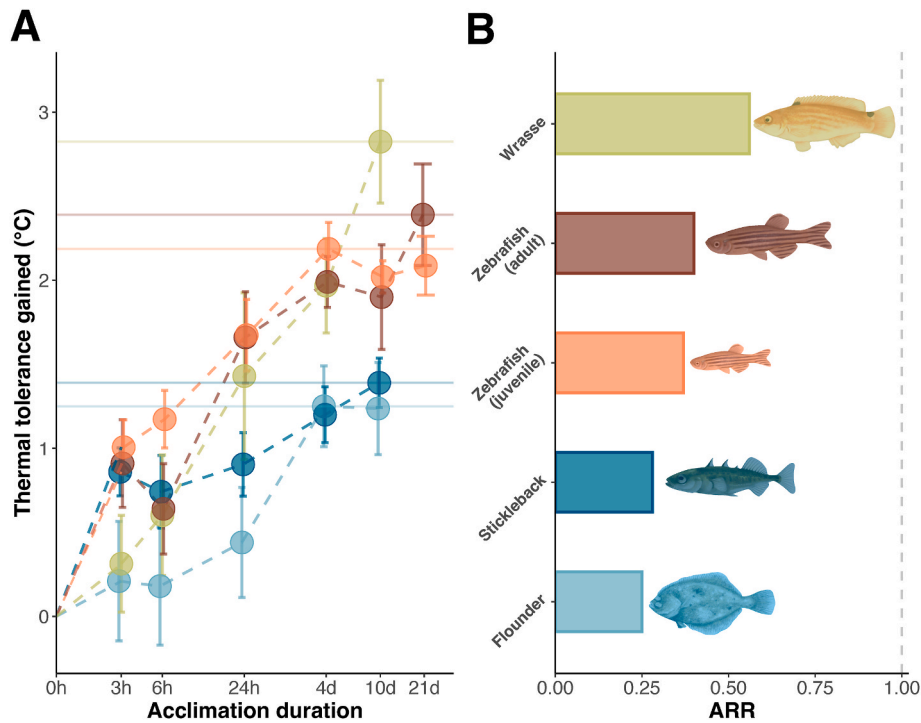


Fig. 4. (A) Thermal tolerance gained (TT_{gain}) through 10 days of acclimation to a warm treatment for species from experiment 1 and up to 21 days for experiment 2 (zebrafish). The mean TT_{gain} for each species is represented as a point at each acclimation duration. Points are slightly jittered horizontally to reduce overlap. Error bars indicate 95% confidence intervals of means. Dotted lines between the means were added for visual purposes. Horizontal lines indicate the maximum TT_{gain} for each species. Acclimation duration is displayed on a logarithmic scale to help visualize differences in time among categorical groups. (B) ARR shown for the different species and life stages tested. ARR was calculated by using the maximum thermal tolerance gained. The dotted line at 1.00 represents a perfect thermal compensation.

with climate change.

Sticklebacks seem to exhibit differences in thermal tolerance based on their population and thermal history. Different marine and freshwater stickleback populations acclimated to a range of temperatures from 16 to 18 °C had similar CT_{max} values, averaging around 30–32 °C (Barrett et al., 2011; Metzger et al., 2016; Mottola et al., 2022). These were all slightly lower than what we observed in our population, which averaged 32.84 °C, when acclimated to 18 °C. To our knowledge, only one previous study evaluated the acclimation capacity of a stickleback to a warm treatment, exposing three-spined stickleback to a +10 °C treatment (26 °C) for 7 days (Mottola et al., 2022) and resulting in an ARR of 0.32, similar to the one obtained here (0.28). Compared to other work published, stickleback in our study had the highest average CT_{max} after 10 days of warming acclimation (34.23 °C). Taken together, our data suggest that marine stickleback could have a thermal “concrete ceiling” around 34 °C for CT_{max} .

We found that some species had a rapid thermal tolerance gain after being exposed to a warm temperature. There was a significant increase in CT_{max} after just 3 h for stickleback and 6 h for wrasse, yet CT_{max} of flounders did not significantly increase until after 4 d of acclimation, which is also the time at which they reached full acclimation. In their study, Stewart et al. (2023) found warm-induced increases in CT_{max} of brook trout in the first 24 h, which was the first time point they tested. For sheepshead minnow (Fangue et al., 2014), the response induction time seemed to be between 12 and 36 h, although $t_{\text{induction}}$ was not explicitly stated. The pupfish (*Cyprinodon dearborni*) demonstrated a $t_{\text{induction}}$ to a warm treatment as early as 3 h (Chung, 1980), similar to our stickleback, while catfish have shown a $t_{\text{induction}}$ of 7 h (Bennett et al., 1998). The present and previous findings highlight the need to assess acclimation over short durations and indicate potential phylogenetic and/or ecological differences in acclimation strategies. Unfortunately, very few studies report the $t_{\text{induction}}$ and we encourage researchers to do so in order to grow the body of knowledge on how

species adjust their physiology when faced with extreme climatic events.

Fishes residing in shallower environments with a high thermal homogeneity and which contain fewer thermal refuges might have less access to cold refugia, potentially increasing the strength of selection on rapid plasticity compared with thermally diverse environments (Einum and Burton, 2023). These fishes may have faster $t_{\text{induction}}$ to react to rapid changes, compared to fishes that can seek refuges. Rapidly increasing their thermal tolerance could be an acclimation strategy employed by sticklebacks, as they live in shallow areas and are exposed to higher average temperatures than wrasses (Fangue et al., 2014). Although flounders also live in shallow areas, our results suggest they might rely on strategies other than rapid thermal tolerance plasticity. Flatfishes often bury themselves in the sand to reduce predation risk and increase prey capture opportunities (Kristensen et al., 2014). This strategy could also be used to help them thermoregulate, as observed in winter flounder (*Pseudopleuronectes americanus*), which had an internal temperature lower than the surrounding water temperature when hiding in sand (Ziegler and Frisk, 2019). Thus, it might not be necessary for them to engage an acclimation response immediately when exposed to increasing temperatures. Our study shows that short exposure to a warm treatment induces a rapid acclimation response for thermal tolerance in most species, but that some species, such as the flounder and wrasse, do not respond as strongly. This hints that certain fish species have a superior ability to cope with fast changing water temperatures, engaging acclimation mere hours following exposure to a change in temperature. Species that have a longer $t_{\text{induction}}$ could be more vulnerable than other species at the onset of a heat wave.

The t_{steady} appears to vary across teleost fishes (Chung, 1980; Fangue et al., 2014), which may be due to differences in phylogeny, ecology, populations, thermal history, holding conditions and intrinsic acclimation mechanisms. Yet, what is clear is that species seem differently equipped to acclimate rapidly to changing environments (Fangue et al., 2014). Species in our study were observed to complete acclimation of

thermal tolerance relatively quickly, with the flounder, stickleback and zebrafish reaching full acclimation within 4 days. Similar times were also observed in pupfish (*Cyprinodon dearborni*), which acclimated to a 7 °C increase in 3 days (Chung, 1980). For other fish species however, time to full acclimation can be longer. For instance, after 10 days, the CT_{max} for the wrasse had not stabilized, suggesting that they had not yet acclimated to this +5 °C change. It was hypothesized that the selection pressure to acclimate as fast as possible is reduced for animals exposed to thermally stratified environments (Einum and Burton, 2023). Goldsinny wrasse and potentially flounder (Ziegler and Frisk, 2019) may be able to rely on behavioural thermoregulation rather than rapid plasticity by exploiting thermal stratification across their deeper depth ranges (or cooler substrata in flounder), compared with the stickleback which resides in shallow (<10m) water (Baldock and Dipper, 2023). Thus, it could be more costly for wrasse and flounder to acclimate as they have evolved to simply move to another habitat or bury when conditions are not optimal (Kristensen et al., 2014; Ziegler and Frisk, 2019). The differences in acclimation dynamics among fishes could thus stem from evolutionary adaptation to their respective thermal niche. Although knowing fish acclimation capacity is relevant for determining thermal limits of species, estimating $t_{induction}$ and t_{steady} helps identify which species are more vulnerable at the onset of a heat wave, and which can acclimate quickly enough to endure their lasting effects.

Contrary to our predictions, we found negligible differences in acclimation dynamics between adult and juvenile zebrafish when assessing upper thermal tolerance. Both life stages had a $t_{induction}$ of 3 h, reached a plateau (t_{steady}) in their CT_{max} in 4 days and had similar TT_{gain} and ARR. The thermal tolerance and the acclimation capacity we observed closely resemble those obtained after a month-long acclimation to the same temperatures by Morgan et al. (2022), suggesting that zebrafish can acclimate to this warming as rapidly as 4 days. The rapid t_{steady} and the lack of differences between life stages could be because both juvenile and adult zebrafish coexist in stagnant tropical shallow waters (Suriyampola et al., 2016) and potentially evolved to cope with rapidly changing temperatures (Morgan et al., 2020). Compared to the species tested in Experiment 1, zebrafish showed similar acclimation dynamics to the stickleback, strengthening the idea that species from shallow, thermally variable environments potentially have faster acclimation rates to react to rapid temperature changes.

We also did not observe any difference between life stages for t_{steady} and $t_{induction}$ of CT_{min} . However, ARR was higher in juveniles, suggesting that adults may be more tolerant to cold, and that warm acclimation incurs a greater trade-off for cold tolerance in juveniles. Both upper and lower thermal tolerance can be size-dependent in ectotherms (Recsetar et al., 2012; Leiva et al., 2019; Gunderson, 2024). Out of 7 tropical fish tested, only the redlight goby (*Coriphopterus urosphilus*) showed a negative relationship between body size and CT_{min} , and none for CT_{max} (Ospina and Mora, 2004), suggesting that the relationship between body size and thermal tolerance is probably highly species-specific for lower (Ospina and Mora, 2004) and upper (Recsetar et al., 2012) thermal tolerance. Here, we found that only CT_{min} was negatively correlated to fish mass and only for juveniles: larger individuals could tolerate lower temperatures. The cause of this life-stage effect on CT_{min} is unknown. One hypothesis could be that this difference is associated with physiological changes due to maturation and reproduction. Alternatively, it has been suggested that small fish may be more vulnerable to low temperature-induced osmotic stress than larger fish, due to the allometric relationship between gill surface area and body mass (Hurst, 2007; Reid et al., 2022).

Although it was hypothesized by Rohr et al. (2018) that larger organisms acclimate slower, we found that the interaction between mass and acclimation duration on CT_{max} and CT_{min} was not significant for most species tested here. This interaction was only significant in flounder, suggesting that larger flounders acclimate slower than smaller individuals, as their CT_{max} at 4 and 10 days of acclimation did not increase as much as it did for smaller fish (Fig. S1). As interactions were

not significant for the other species, the relationship between mass and acclimation rate is likely species-specific (Bennett et al., 1998; Einum and Burton, 2023).

Compared to CT_{max} , both the t_{steady} and the $t_{induction}$ were slower for CT_{min} , indicating that zebrafish adjust their upper thermal tolerance at a faster rate than their lower thermal tolerance at the same warm acclimation temperature. This could arise from different mechanisms between initial and later acclimation and suggests that the mechanisms for rapid warm acclimation do not come at the cost of cold tolerance, whereas later warm acclimation responses negatively affect cold tolerance. Another possibility is that the dynamics of CT_{max} vs CT_{min} plasticity depend on the proximity of acclimation temperature to the upper or lower thermal limits; perhaps, the rate of CT_{max} plasticity is prioritized at warm acclimation temperatures whereas CT_{min} plasticity is prioritized at cold acclimation temperatures. It has been suggested that due to smaller intraspecific variability in tropical fishes than temperate fishes, the former were more vulnerable to thermal stress (Nati et al., 2021). Yet, we showed that zebrafish have a fast $t_{induction}$, a fast t_{steady} and a relatively high ARR compared to other temperate species tested here, and elsewhere (Gunderson and Stillman, 2015; Morley et al., 2019; Ruthsatz et al., 2024). This supports the idea of Gunderson and Stillman (2015) that although tropical species could be more at risk to warming than temperate species, potentially living closer to their upper thermal limit, it is not due to a lack in thermal plasticity. Studies on thermal tolerance of zebrafish are rapidly multiplying (Vergauwen et al., 2013; Morgan et al., 2018; Åsheim et al., 2020), cementing their importance as a model organism in many fields of biological research (Briggs, 2002; López-Olmeda and Sánchez-Vázquez, 2011; Morgan et al., 2019). Our study highlights their impressively fast acclimation dynamics, which can guide future work on this species.

5. Conclusion and perspectives

Our results demonstrate species-specific acclimation dynamics of upper and lower thermal tolerance among fishes, and support the notion that interspecific differences in acclimation dynamics stem from acclimation strategies evolved in response to species-specific ecology. Understanding these interspecific differences can improve our knowledge of species resilience to extreme weather events. While thermal plasticity of fishes is well documented, our study helps fill the existing gap on the detailed short-term time-course of plasticity, such as the response induction time ($t_{induction}$) and the time to full acclimation (t_{steady}) (Einum and Burton, 2023). Here, we defined the important temporal attributes of thermal plasticity of $t_{induction}$ and t_{steady} , which complement established measures of acclimation capacity (ARR and TT_{gain}). We chose to acclimate different species to different temperatures in order to better reflect their natural conditions and likely warming-scenarios. This trades off with comparability between species and we recommend that the observed differences between species are interpreted with this in mind. Nonetheless, generating data on acclimation dynamics could benefit species distribution models and climate risk assessments by improving their ecological relevance and allowing them to be tailored to the acclimation dynamics of the studied species (Burton et al., 2022). Here, we observed differences in the potential for rapid acclimation between species, underscoring the need for thermal biologists to consider acclimation dynamics in experimental designs, especially for studies comparing acclimation capacities of multiple species. This approach would increase the relevance of interspecific comparisons within a broader ecological framework. Additionally, it would be worthwhile to explore underlying mechanisms of acclimation, such as gill remodeling, mitochondria efficiency and comparing HSP gene expression across multiple acclimation durations. Lastly, an important future direction is to evaluate the extent to which differences in ecology and previous thermal history contribute to explaining interspecific differences in acclimation dynamics and capacities.

CRedit authorship contribution statement

Jeremy De Bonville: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Anna H. Andreassen:** Writing – review & editing, Investigation. **Zara-Louise Cowan:** Writing – review & editing, Investigation. **Lorena Silva-Garay:** Writing – review & editing, Investigation. **Robine H.J. Leeuwis:** Writing – review & editing, Investigation. **Eirik R. Åsheim:** Writing – review & editing, Investigation, Formal analysis. **Ben Speers-Roesch:** Writing – review & editing, Investigation, Conceptualization. **Graham D. Raby:** Writing – review & editing, Investigation, Conceptualization. **Sandra A. Binning:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Fredrik Jutfelt:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Ethical approvals

Experimental procedures were approved by the Ethical Committee for Animal Research in Gothenburg (permit #5.8.18–8955/2022) and by the Norwegian Food Safety Authority (permit #29878).

Data availability

Data and code used in this study are publicly archived and were made available to editors and reviewers at the time of submission: <https://doi.org/10.6084/m9.figshare.26356939.v1>.

Funding sources

This work was supported by the Outstanding Academic Fellows Programme (OAFP) grant, NTNU (F.J.) and the European Research Council Consolidator grant (CLIMEVOLVE, to F.J.). J.D.B. was supported by travel grants from the Company of Biologists (COB) and the Groupe de Recherche Interuniversitaire en Limnologie (GRIL). S.A.B, G. D.R and B.S.-R. are supported by the NSERC Discovery Grant Program.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the members of the FIN Club 2022, 2023; 2024 who assisted with fish collection, feeding and discussing ideas. We are thankful to Anders Meyer for assisting with CT_{max} and CT_{min} trials, to Eline Rypdal for assisting with fish care at NTNU and to Andréa Serres who conducted preliminary statistical analyses. Fish illustrations by Victoria Thelamon. We are thankful to the two reviewers who provided insightful comments. This is FIN Club paper #12.

Appendix A. Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2024.104024>.

References

- Abram, P.K., Boivin, G., Moiroux, J., Brodeur, J., 2017. Behavioural effects of temperature on ectothermic animals: unifying thermal physiology and behavioural plasticity. *Biol. Rev.* 92, 1859–1876.
- Amat-Trigo, F., Andreou, D., Gillingham, P.K., Britton, J.R., 2023. Behavioural thermoregulation in cold-water freshwater fish: innate resilience to climate warming? *Fish Fish.* 24, 187–195.

- Angiletta, M.J., 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, New York, USA.
- Åsheim, E.R., Andreassen, A.H., Morgan, R., Jutfelt, F., 2020. Rapid-warming tolerance correlates with tolerance to slow warming but not growth at non-optimal temperatures in zebrafish. *J. Exp. Biol.* 223. <https://doi.org/10.1242/jeb.229195>.
- Baldock, L., Dipper, F., 2023. *Inshore Fishes of Britain and Ireland*. Princeton University Press, Princeton, USA.
- Barrett, R.D.H., Paccard, A., Healy, T.M., Bergek, S., Schulte, P.M., Schluter, D., Rogers, S.M., 2011. Rapid evolution of cold tolerance in stickleback. *Proc R Soc B Biol Sci* 278, 233–238.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67 (1), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Beitinger, T.L., Bennett, W.A., McCauley, R.W., 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environ. Biol. Fish.* 58, 237–275.
- Bennett, W.A., McCauley, R.W., Beitinger, T.L., 1998. Rates of gain and loss of heat tolerance in channel catfish. *Trans. Am. Fish. Soc.* 127, 1051–1058.
- Blewett, T.A., Binning, S.A., Weinrauch, A.M., Ivy, C.M., Rossi, G.S., Borowiec, B.G., Lau, G.Y., Overduin, S.L., Aragao, I., Norin, T., 2022. Physiological and behavioural strategies of aquatic animals living in fluctuating environments. *J. Exp. Biol.* 225. <https://doi.org/10.1242/jeb.242503>.
- Brett, J.R., 1970. Environmental factors, part 1. Temperature. In: Kinne, O. (Ed.), *Marine Ecology*, ume I. Wiley, London, pp. 513–560.
- Briggs, J.P., 2002. The zebrafish: a new model organism for integrative physiology. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 282, R3–R9.
- Burton, T., Lakka, H.K., Einum, S., 2020. Acclimation capacity and rate change through life in the zooplankton *Daphnia*. *Proc R Soc B Biol Sci* 287. <https://doi.org/10.1098/rspb.2020.0189>.
- Burton, T., Ratikainen, I.I., Einum, S., 2022. Environmental change and the rate of phenotypic plasticity. *Global Change Biol.* 28, 5337–5345.
- Chung, K.S., 1980. Rate of acclimation of the tropical saltmarsh fish *Cyprinodon dearborni* to temperature changes. *Hydrobiologia* 78, 177–181.
- Claussen, D.L., 1977. Thermal acclimation in ambystomatid salamanders. *Comp. Biochem. Physiol. Part A Physiol.* 58, 333–340.
- Currie, R.J., Bennett, W.A., Beitinger, T.L., 1998. Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environ. Biol. Fish.* 51, 187–200.
- De Bonville, J., Côté, A., Binning, S.A., 2024. Thermal tolerance and survival are modulated by a natural gradient of infection in differentially acclimated hosts. *Conserv Physiol* 12, coae015.
- Desforges, J.E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K.M., Eliason, E.J., Dressler, T.L., McKenzie, D.J., Bates, A.E., Lawrence, M.J., Fanguie, N., et al., 2023. The ecological relevance of critical thermal maxima methodology (CTM) for fishes. *J. Fish. Biol.* 1000–1016.
- Doudoroff, P., 1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (ayres). *Biol. Bull.* 83, 219–244.
- Dufty, A.M., Clobert, J., Møller, A.P., 2002. Hormones, developmental plasticity and adaptation. *Trends Ecol. Evol.* 17, 190–196.
- Einum, S., Burton, T., 2023. Divergence in rates of phenotypic plasticity among ectotherms. *Ecol. Lett.* 26, 147–156.
- Einum, S., Ratikainen, I., Wright, J., Pélabon, C., Bech, C., Jutfelt, F., Stawski, C., Burton, T., 2019. How to quantify thermal acclimation capacity? *Global Change Biol.* 25, 1893–1894.
- Ern, R., Andreassen, A.H., Jutfelt, F., 2023. Physiological mechanisms of acute upper thermal tolerance in fish. *Physiology* 38, 141–158.
- Fanguie, N.A., Wunderly, M.A., Dabruzzi, T.F., Bennett, W.A., 2014. Asymmetric thermal acclimation responses allow sheepshead minnow *cyprinodon variegatus* to cope with rapidly changing temperatures. *Physiol. Biochem. Zool.* 87, 805–816.
- Fischer, B., van Doorn, G.S., Dieckmann, U., Taborsky, B., 2014. The evolution of age-dependent plasticity. *Am. Nat.* 183, 108–125.
- Fu, K.K., Fu, C., Qin, Y.L., Bai, Y., Fu, S.J., 2018. The thermal acclimation rate varied among physiological functions and temperature regimes in a common cyprinid fish. *Aquaculture* 495, 393–401.
- Gunderson, A.R., 2024. Disentangling physiological and physical explanations for body size-dependent thermal tolerance. *J. Exp. Biol.* 227. <https://doi.org/10.1242/jeb.245645>.
- Gunderson, A.R., Stillman, J.H., 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc R Soc B Biol Sci* 282. <https://doi.org/10.1098/rspb.2015.0401>.
- Healy, T.M., Schulte, P.M., 2012. Factors affecting plasticity in whole-organism thermal tolerance in common killifish (*Fundulus heteroclitus*). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 182, 49–62.
- Hurst, T.P., 2007. Causes and consequences of winter mortality in fishes. *J. Fish. Biol.* 71, 315–345.
- IPCC, 2022. *Climate Change 2022: Impacts, Adaptation and Vulnerability*. Cambridge University Press.
- Jutfelt, F., Ern, R., Leeuwis, R.H.J., Clark, T.D., 2024. Effects of climate warming. In: *Encyclopedia of Fish Physiology*, second ed. Edition. Elsevier, Second Edition.
- Komoroske, L.M., Connon, R.E., Lindberg, J., Cheng, B.S., Castillo, G., Hasenbein, M., Fanguie, N.A., 2014. Ontogeny influences sensitivity to climate change stressors in an endangered fish. *Conserv Physiol* 2, 1–13.
- Kristensen, L.D., Sparrevojn, C.R., Christensen, J.T., Støttrup, J.G., 2014. Cryptic behaviour of juvenile turbot *psetta maxima* L. And European flounder *Platichthys flesus* L. *Open J. Mar. Sci.* 4, 185–193.

- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. *J. Stat. Software* 82 (13), 1–26. <https://doi.org/10.18637/jss.v082.i13>.
- Leeuwis, R.H.J., Gamperl, A.K., 2022. Adaptations and plastic phenotypic responses of marine animals to the environmental challenges of the high intertidal zone, 1st editio. In: *Oceanography and Marine Biology: an Annual Review*, Edition, 60. CRC press.
- Leiva, F.P., Calosi, P., Verberk, W.C.E.P., 2019. Scaling of thermal tolerance with body mass and genome size in ectotherms: a comparison between water- and air-breathers. *Philos Trans R Soc B Biol Sci* 374. <https://doi.org/10.1098/rstb.2019.0035>.
- Lenth, R., 2023. Emmeans: estimated marginal means, aka least-squares means. Rpackageversion1.8.5. <https://CRAN.R-project.org/package=emmeans>.
- Loeb, J., Wasteneys, H., 1912. On the adaptation of fish (*Fundulus*) to higher temperatures. *J. Exp. Zool.* 12, 543–557.
- López-Olmeda, J.F., Sánchez-Vázquez, F.J., 2011. Thermal biology of zebrafish (*Danio rerio*). *J. Therm. Biol.* 36, 91–104.
- Lubitz, N., Daly, R., Smoothey, A.F., Vianello, P., Roberts, M.J., Schoeman, D.S., Sheaves, M., Cowley, P.D., Dagorn, L., Forget, F.G., et al., 2024. Climate change-driven cooling can kill marine megafauna at their distributional limits. *Nat. Clim. Change* 14, 526–535.
- McAcy, B.N., 2015. Physiological Adaptations of Diamond Killifish (*Adinia Xenica*) to Survive Temperature Extremes (Masters Thesis). University of West Florida.
- Metzger, D.C.H., Healy, T.M., Schulte, P.M., 2016. Conserved effects of salinity acclimation on thermal tolerance and hsp70 expression in divergent populations of threespine stickleback (*Gasterosteus aculeatus*). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 186, 879–889.
- Morash, A.J., Speers-Roesch, B., Andrew, S., Currie, S., 2021. The physiological ups and downs of thermal variability in temperate freshwater ecosystems. *J. Fish. Biol.* 98, 1524–1535.
- Morgan, R., Andreassen, A.H., Åsheim, E.R., Finnøen, M.H., Dresler, G., Brembu, T., Loh, A., Miest, J.J., Jutfelt, F., 2022. Reduced physiological plasticity in a fish adapted to stable temperatures. *Proc. Natl. Acad. Sci. U. S. A.* 119. <https://doi.org/10.1073/pnas.2201919119>.
- Morgan, R., Finnøen, M.H., Jensen, H., Pélabon, C., Jutfelt, F., 2020. Low potential for evolutionary rescue from climate change in a tropical fish. *Proc. Natl. Acad. Sci. U. S. A.* 117, 33365–33372.
- Morgan, R., Finnøen, M.H., Jutfelt, F., 2018. CTmax is repeatable and doesn't reduce growth in zebrafish. *Sci. Rep.* 8, 1–8.
- Morgan, R., Sundin, J., Finnøen, M.H., Dresler, G., Vendrell, M.M., Dey, A., Sarkar, K., Jutfelt, F., 2019. Are model organisms representative for climate change research? Testing thermal tolerance in wild and laboratory zebrafish populations. *Conserv. Physiol.* 7, 1–11.
- Morley, S.A., Peck, L.S., Sunday, J.M., Heiser, S., Bates, A.E., 2019. Physiological acclimation and persistence of ectothermic species under extreme heat events. *Global Ecol. Biogeogr.* 28, 1018–1037.
- Mottola, G., López, M.E., Vasemägi, A., Nikinmaa, M., Anttila, K., 2022. Are you ready for the heat? Phenotypic plasticity versus adaptation of heat tolerance in three-spined stickleback. *Ecosphere* 13, 1–14.
- Nati, J.J.H., Svendsen, M.B.S., Marras, S., Killen, S.S., Steffensen, J.F., McKenzie, D.J., Domenici, P., 2021. Intraspecific variation in thermal tolerance differs between tropical and temperate fishes. *Sci. Rep.* 11, 1–8.
- O'Dea, R.E., Noble, D.W.A., Nakagawa, S., 2022. Unifying individual differences in personality, predictability and plasticity: a practical guide. *Methods Ecol. Evol.* 13, 278–293.
- Ospina, A.F., Mora, C., 2004. Effect of body size on reef fish tolerance to extreme low and high temperatures. *Environ. Biol. Fish.* 70, 339–343.
- R Core Team, 2022. R: A Language and Environment for Statistical Computing. RFoundationforStatisticalComputing, Vienna, Austria. <https://www.R-project.org/>.
- Recsetar, M.S., Zeigler, M.P., Ward, D.L., Bonar, S.A., Caldwell, C.A., 2012. Relationship between fish size and upper thermal tolerance. *Trans. Am. Fish. Soc.* 141, 1433–1438.
- Reid, C.H., Patrick, P.H., Rytwinski, T., Taylor, J.J., Willmore, W.G., Reesor, B., Cooke, S.J., 2022. An updated review of cold shock and cold stress in fish. *J. Fish. Biol.* 100, 1102–1137.
- Rohr, J.R., Civitello, D.J., Cohen, J.M., Roznik, E.A., Sinervo, B., Dell, A.I., 2018. The complex drivers of thermal acclimation and breadth in ectotherms. *Ecol. Lett.* 21, 1425–1439.
- Ruthsatz, K., Dahlke, F., Alter, K., Wohlrab, S., Eterovick, P.C., Lyra, M.L., Gippner, S., Cooke, S.J., Peck, M.A., 2024. Acclimation capacity to global warming of amphibians and freshwater fishes: drivers, patterns, and data limitations. *Global Change Biol.* 30, 1–20.
- Stewart, E.M.C., Frasca, V.R., Wilson, C.C., Raby, G.D., 2023. Short-term acclimation dynamics in a coldwater fish. *J. Therm. Biol.* 112, 103482.
- Sundin, J., Morgan, R., Finnøen, M.H., Dey, A., Sarkar, K., Jutfelt, F., 2019. On the observation of wild zebrafish (*Danio rerio*) in India. *Zebrafish* 16, 546–553.
- Suriyampola, P.S., Shelton, D.S., Shukla, R., Roy, T., Bhat, A., Martins, E.P., 2016. Zebrafish social behavior in the wild. *Zebrafish* 13, 1–8.
- Tarapacki, P., Jørgensen, L.B., Sørensen, J.G., Andersen, M.K., Colinet, H., Overgaard, J., 2021. Acclimation, duration and intensity of cold exposure determine the rate of cold stress accumulation and mortality in *Drosophila suzukii*. *J. Insect Physiol.* 135. <https://doi.org/10.1016/j.jinsphys.2021.104323>.
- Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., McCann, K.S., Savage, V., Tunney, T.D., O'Connor, M.I., 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proc R Soc B Biol Sci* 281. <https://doi.org/10.1098/rspb.2013.2612>.
- Vergauwen, L., Knapen, D., Hagenaars, A., De Boeck, G., Blust, R., 2013. Assessing the impact of thermal acclimation on physiological condition in the zebrafish model. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 183, 109–121.
- Vinagre, C., Mendonça, V., Cereja, R., Abreu-Afonso, F., Dias, M., Mizrahi, D., Flores, A.A.V., 2018. Ecological traps in shallow coastal waters-Potential effect of heat-waves in tropical and temperate organisms. *PLoS One* 13, 1–17.
- Wilbur, N.M., O'Sullivan, A.M., MacQuarrie, K.T.B., Linnansaari, T., Curry, R.A., 2020. Characterizing physical habitat preferences and thermal refuge occupancy of brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) at high river temperatures. *River Res. Appl.* 36, 769–783.
- Ziegler, C.M., Frisk, M.G., 2019. Flatfish utilize sediment blanket to facilitate thermoregulation. *Mar. Ecol. Prog. Ser.* 609, 179–186.