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Thermal tolerance limits and physiological traits as indicators of *Hediste diversicolor*'s acclimation capacity to global and local change drivers

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ABSTRACT

Global projections predict significant increases in ocean temperature and changes in ocean chemistry, including salinity variations by 2100. This has led to a substantial interest in the study of thermal ecophysiology, as temperature is a major factor shaping marine ectotherm communities. However, responses to temperature may be influenced by other factors such as salinity, highlighting the relevance of multiple stressor studies. In the present work, we experimentally evaluated the thermal tolerance of the marine ragworm *Hediste diversicolor* under predicted global change scenarios. Organisms were subjected to an experimental trial under control (24 °C), and two temperature treatment scenarios (ocean warming +3 °C – (27 °C) and heat wave +6 °C – (30 °C)), combined with salinity variations (20 and 30) in a full factorial design for 29 days. Environmental data from the field were collected during 2019 and 2020. At day 30 post exposure, upper thermal limits (Critical Thermal Maximum - CTMax), thermal safety margins (TSM) and acclimation capacity were measured. Higher acclimation temperatures led to higher thermal tolerance limits, confirming that *H. diversicolor* features some physiological plasticity, acclimation capacity and a positive thermal safety margin. This margin was greater considering *in situ* temperature data from 2019 than maximum temperatures for 2020 (CTMax > maximum habitat temperature–MHT). Moreover, smaller organisms displayed higher upper thermal limits suggesting that thermal tolerance is size dependent. Ragworms subjected to higher salinity also showed a higher CTMax than those acclimated to lower salinity. However, temperature and salinity showed an additive effect on CTMax, as no significant interaction was detected. We conclude that *H. diversicolor* can easily acclimate to increased water temperature, independently of salinity variations. Given the key role of ragworms in food webs in estuaries and coastal lagoons, substrate bioturbation and aquaculture, this information is relevant to support conservation actions, optimize culture protocols and identify thermal resistant strains.

1. Introduction

The thermal tolerance window is the favourable range of temperature or performance breadth of a given species (Madeira et al., 2012; Velasco-Blanco et al., 2019). Climate change increased the scientific community's interest in understanding species' thermal limits, enabling to predict how species will react if this limit is overcome (Angilletta et al., 2002; Peck et al., 2014; Vinagre et al., 2016; Sunday et al., 2019).

Species from intertidal or tropical environments live close to their upper acute thermal tolerance limit, being more susceptible to deleterious effects promoted by global warming (Tewksbury et al., 2008; Madeira et al., 2012; Vinagre et al., 2016). Nevertheless, a considerable lack of knowledge on thermal limits of marine organisms remains, especially how multiple environmental factors (e.g. salinity, hypoxia, pollution) modulate thermal tolerance limits of marine species. Understanding if organisms can tolerate new thermal variations in their habitat and environment is only a small part of global change research, being essential to understand the response of populations and communities to increasing temperatures and extreme weather events, such as marine heatwaves. As oceans warm and marine heatwaves (MHW)

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expand (e.g., by affecting bigger areas at the same time) (Laufkötter et al., 2020), become more frequent (Coleman and Wernberg 2020), intense and long-lasting (IPCC, 2022), temperate organisms will most likely face both chronic and acute thermal stress.

Organisms can have different responses to warmer temperatures: diffuse towards more favourable habitats, cope with new conditions through phenotypic and physiological plasticity (Fox et al., 2019), or adapt through genetic changes (Hofmann 2005; Gibbin et al., 2017). Regarding thermal acclimation, phenotypic plasticity can be any phenotypic alteration (usually reversible) in physiology promoted by environmental temperature that modifies organisms' performance, possibly improving their fitness (Chown et al., 2009; Massamba-N'Siala et al., 2014). On the opposite, some phenotypic plasticity responses are non-reversible and remain throughout the organism's life cycle, leading to carry over effects that determine fitness outcomes (Slotsbo et al., 2016; Moore and Martin 2019). However, since long-term acclimation in a long-term imposes energy costs to an organism in terms of growth and reproduction (Angilletta 2009), this aspect should be taken into consideration in species vulnerability assessments, as it ultimately determines the success and viability of populations enduring global change. Moreover, smaller organisms are expected to have a reduced mismatch between oxygen demand and tissue oxygenation capacity when compared to larger organisms, showing greater whole-organism thermal tolerance (Pörtner et al., 2017); consequently smaller specimens may eventually benefit more quickly from natural selection. As smaller organisms display faster acclimation, this could also result in a greater thermal windows than that displayed by larger organisms, since smaller organisms can sustain higher performances over wider temperature ranges (Rohr et al., 2018). The potential effects on organismal performance can escalate if additional stressors, (e.g., salinity variations), interact with temperature, potentially threatening the future of marine communities. In addition, the interaction between two, or more, stressors may lead to additive, antagonistic or synergistic responses by organisms (Côté et al., 2016; Jackson et al., 2021). In marine species, stress caused by sub or supra saline environments, interacting with warmer waters which, by itself, already increase organisms' metabolic rate, may demand larger amounts of metabolic energy to be allocated to re-establish the organism's homeostasis than saline or thermal stress acting individually (Spanopoulos-Hernández et al., 2005; Jackson et al., 2021).

To determine the thermal tolerance of a species, contrasting experimental approaches are frequently used: 1) the determination of Lethal Temperature, i.e. the temperature that causes a mortality of 50% of the individuals in a given sample, (Somero 2011); and 2) the determination of the Critical Thermal Maximum (CTMax), i.e. the temperature that initiates the loss of motor function, with this feature being determined by the constant increase of temperature to reach a critical point (Mora and Ospína 2001; Madeira et al., 2012; Massamba-N'Siala et al., 2012; Missionário et al., 2022). The second method is more commonly used in thermal biology research on this topic, as it is easier to apply, faster and requires smaller sample sizes (Morgan et al., 2018). Furthermore, among ectothermic vertebrates and invertebrates, it is generally accepted that CTMax is the most efficient index of upper thermal tolerance (Lutterschmidt and Hutchison 1997).

Hediste diversicolor is a benthic polychaete species that belongs to the Nereididae family (Müller, 1776). It inhabits estuaries and coastal lagoons, where it is influenced by the tides along the temperate North Atlantic coast (Scaps 2002). This species is euryoecious and euryhaline, adapting to a wide range of environmental conditions, (e.g., hypoxia and low salinities). *Hediste diversicolor* is a gonochoristic species, with maturation occurring between the ages of one and three years old. However, since this species is ectothermic, warmer temperatures can accelerate sexual maturation. As prey, this species is key in temperate trophic webs, being predated upon by a large number of fish, crustacean and bird species. (Scaps 2002; Gillet and Torresani 2003; Nesto et al., 2012). In addition to its ecological relevance, the ragworms hold high commercial value as bait for sports fishing and effluent bioremediation in integrated multi-trophic aquaculture (IMTA), due to their ability to reuse organic matter available in the effluent waters of fish farm (Marques et al., 2018; Wang et al., 2019; Jerónimo et al., 2021).

The aim of the present study was to investigate the acclimation capacity of *H. diversicolor* under different global (temperature) and local (salinity) change scenarios to infer on its capacity to withstand ocean warming and MHW events. For that purpose, we maintained individuals under experimental controlled conditions of temperature and salinity to determine their upper thermal tolerance limits, thermal safety margins and acclimation capacity ratio. To have a more realistic view of thermal regimes *in situ,* we collected temperature data over two consecutive years (2019 and 2020). To evaluate potential links between thermal tolerance and weight, we tested the correlation between *H. diversicolor* upper thermal limits and wet weight. We hypothesize that higher acclimation temperatures promote higher thermal tolerance limits, given the physiological plasticity of eurythermal intertidal species. However, this response may be modulated by salinity variations. Moreover, we hypothesize that smaller organisms have higher thermal limits than bigger organisms, as they are less likely to spend energy on energetically costly processes (such as reproduction) and should be less susceptible to oxygen limitation due to their higher surface area to volume ratio. We also hypothesize that intertidal temperatures vary between the two years, showing relevant interannual variability, affecting thermal safety margins (TSM) estimations. Finally, we highlight the importance of this study, as this estuarine and coastal species plays an important role on ecosystem processes and services, being an important prey item in trophic webs and a major bioturbator in coastal environments.

2. Materials and methods

2.1. Ethical statement

This study was conducted following all relevant EU legislation for animal experiments (Directive, 2010/63/EU), mostly related to and complied with the 3 Rs principles of animal welfare. Although the Directive does not include most invertebrates, many of the principles can be applied to any animal experiment (partial replacement: using invertebrates instead of vertebrates; reduction: the experimental design is robust to ensure reproducible results with the least amount of animals, while maximizing the relevant information to be extracted and promoting data sharing for re-use and analysis; refinement: ensuring the quality and enrichment of the life support systems, promoting the well-being and health of all experimental animals). The last author Diana Madeira is licensed by the Portuguese National Authority for Food and Animal Health to carry out animal experiments, holding a C level certification by the Federation of European Laboratory Animal Science Association.

2.2. Field work and animal husbandry

Field temperature data was measured between July 2019 and October 2020 at Ria de Aveiro, namely in Espinheiro channel (40°38′2″N, 8°39′42″W, Fig. S1), to determine seasonal, within season and daily (tide cycle) fluctuations in the ragworms' habitat. Water temperature was registered every 30 min using a data logger (HOBO® Water Temp Pro v2 U22 – 001, Onset, USA). Moreover, salinity and pH measurements were taken during 2019, at a frequency of once a week amidst low tide. To assess tidal cycle variations, we also recorded salinity and pH during a full tide cycle at a frequency of once per month between sunrise and sunset, using a WTW® multiparameter probe (Xylem Analytics, Germany).

Organisms (n = 1470, weight 0.22 g ± 0.08 g, measured in a subsample of 60 individuals) were collected from Espinheiro channel at Ria de Aveiro (40°38′2″N, 8°39′42″W, Fig. S1) in 2018 (autumn season)

amidst low tide. During specimen collection, the average water temperature was 15 °C and the average salinity was 25 (measured with a conductivity portable meter 3110 SET 1 WTW® probe). During the transport to laboratory, organisms were placed in buckets containing macroalgae.

Organisms were housed in 47L-plastic containers for two weeks containing artificial seawater and a sediment layer made of sand from WhiteMinerals® (0.5 – 0.7 mm) to simulate ragworms natural environment. Containers were supplied with continuous aeration. Artificial seawater was prepared by mixing reverse osmosis water and Red Sea® salt crystals (25 salinity – mimicking the environmental conditions when the organisms were sampled). The temperature of the water was kept at 15 ± 0.5 °C and feed was provided to ragworms every 2 days (flaked commercial fish food - containing 42% protein and 11% fat). The photoperiod cycle was established at 12 h L: 12 h D cycle with white fluorescent lamps placed above the containers.

2.3. Acclimation assay

Following the 2-week housing timeframe, organisms were gradually adapted to the temperature treatments by increasing temperature at a rate of (2 °C h^{-1}) (Missionário et al., 2022) to reach the temperature scenarios of control – (24 °C), +3 °C – (27 °C) and +6 °C (30 °C), predicted by the Intergovernmental Panel on Climate Change (IPCC) for 2100 (IPCC, 2019) and adapted to local environmental regimes. During thermal ramps, salinity was maintained at 25. Thermal ramping was performed as described in Madeira et al. (2021), using a water bath in which temperature was controlled by a custom-made digital heater device (Aqualgae Soc Lda, Portugal). Briefly, we used six buckets of 25 L, each containing 81–82 specimens and thermal ramping was performed until we reached the total sample size of 490 ragworms per warming scenario (giving a total of 1470 in the experiment).

Subsequently, organisms were randomly allocated to 6.5-L plastic tanks with different treatments (levels of water temperature - 24 °C, 27 °C and 30 °C- and salinity (20 and 30) in a full factorial design (six treatments in total, n = 7 tanks $treatment^{-1}$, n = 35 specimens $tank^{-1}$, n_{total} per treatment = 245) (Fig. S2.1 and S2.2). Salinity values were based on the range of salinities reported for Ria de Aveiro, namely 20 to 35 (Abrantes et al., 1999). To simulate field conditions, each replicate tank had 80 mm of sand and seawater (prepared as described above). Each replicate was also wrapped in opaque black plastic, to avoid light from entering the sediment column. Organisms were exposed to the six treatments for a period of 29 days. Temperature was kept in each tank using individual Eheim® thermocontrol heaters of 50 W. Oxygen levels were kept using an aeration device (Hailea® vortex blower) with a diffuser stone in each tank. In addition, one 47-L depuration tank per treatment (was kept at the intended temperature with a 100-W heater. Inside each depuration tank were seven 1-L glass flasks containing 40 mm of sand and sea water (aerated), corresponding to the 7 tanks for each treatment (Fig. S2.1 and S2.2).

Ragworms were fed every 2-days with flaked feed (as described above). Water chemistry in each tank (salinity, dissolved oxygen and pH) were monitored using WTW probes for conductivity, oxygen and pH; ammonia and nitrites' levels in the water were monitored using Salifert Profi colorimetric water tests. Approximately 50% of the water in each tank was replaced every week.

2.4. Thermal tolerance limits

The critical upper thermal limit of ragworms was determined following a dynamic method commonly employed in the determination of thermal tolerance in ectothermic animals (Madeira et al., 2012; Kaspari et al., 2015; Vinagre et al., 2016).

To determine the Critical Thermal Maximum (CTMax), after the acclimation assay (at day 30 post exposure) and 24 h of depuration, three different individuals per replicate tank (n = 21 per treatment) were moved to other 1-L glass flasks (with a 5 mm sand bottom, and aerated seawater). These glass flasks were grouped by temperature treatment and assigned to the correct salinity value and randomly positioned inside a water bath (the same as described above) (Figure S3.1 and Fig. S3.2). In summary, three CTMax assays were run, each starting at the tested acclimation temperatures (24, 27 and 30 °C).

During the CTMax assay, individuals were subjected to an ecologically realistic thermal ramp of 2 °C h^{-1}, (Missionário et al., 2022), until they reached the behavioural end-point, defined as loss of equilibrium, i.e. individuals were not in their gravitational position, showing loss of righting response (previously tested in a pilot trial). The CTMax trial was run under continuous observation and behaviour of individuals', such as digging activity (i.e., specimens attempting to burrow or emerged from the substrate) and the response to prodding (i.e., specimens motionless and moving only after being gently prodded with a tip) was also registered, as defined by Massamba-N'Siala et al. (2012). After reaching the endpoint, the wet weight of the specimens was registered to the nearest 0.01 g (Kern EMB 200-3 Scale).

The total number of individuals sampled in the CTmax assay was 126, out of a total of 1470 used in the full experiment. This discrepancy relates to the fact that several other sampling activities were carried out during the experiment to answer different research questions, resulting in other publications (Fernandes et al., 2021; Madeira et al., 2021b; 2021a)).

2.5. Data analyses

Descriptive statistics for temperature data (means and SD, minimum and maximum values) were computed and data were plotted using the software GraphPad Prism v9.0.0.

Data of wet weight was tested for normality (D'Agostino-Pearson normality test) and homoscedasticity (Bartlett's test). A Two-way ANOVA, using temperature and salinity as fixed factors, was performed to test significant differences between wet weight data of the six different treatments after the 29 days of chronic exposure and post-CTMax assay (acute exposure). Tukey's post-hocs were used to test for differences between treatments.

CTMax was calculated as the mean of all end points reached by the individuals (Mora and Ospína 2001; Madeira et al., 2012; Massamba-N'Siala et al., 2012), following the equation:

$$\text{CTMax (treatment)} = \sum(T_{endpoint}\ n) / n,$$

where $T_{endpoint}$ is the temperature at which the endpoint was observed for any given specimen and *n* the number of individuals that were in the treatment.

Coefficients of variation (% CV) were used to calculate intraspecific variation in CTMax, following the equation:

$$\%\ CV = (SD/Mean) \times 100,$$

where SD is the standard deviation of all CTMax individual values and Mean is the respective average of CTMax.

Acclimation capacity was also calculated as:

$$\text{Acclimation capacity (°C)} = \text{CTMax treatment - CTMax control},$$

where CTMax treatment means the CTMax of each treatment group subtracted by CTMax of control group (T24S30).

The thermal safety margin (TSM, i.e. warming tolerance) was estimated as the difference between the average CTMax previously determined and the Maximum Habitat Temperature (MHT), extracted from the temperature data obtained *in situ* (Madeira et al., 2019; Missionário et al., 2022). TSM indicates how near this species is from its critical upper thermal limit.

Acclimation response ratio (ARR) is a proxy for heat tolerance plasticity and is calculated as the change in heat tolerance (ΔCTMax) for a given change in acclimation temperature (ΔT_{acc}) (Claussen 1977):

$$ARR = \frac{\Delta CTMax}{\Delta T_{acc}},$$

An ARR = 0 means no plasticity whereas ARR = 1 means complete physiological compensation for environmental warming.

Exploratory analyses were performed on the dataset to test normality (D'Agostino-Pearson normality test) and homoscedasticity (Bartlett's test). A Two-way ANOVA, using temperature and salinity as fixed factors, was performed to test significant differences between the critical thermal maximum of ragworms subjected to the six different treatments. Box & whiskers graphs were plotted to better visualize the results. Correlation between wet weight - CTMax was examined using Spearman correlation test, as the assumptions of normality and homoscedasticity were not met. Additionally, we considered the mean CTMax and wet weight of three individuals from each replicate since they belonged to the same replicate/tank/experimental unit of each treatment. All tests were performed using the software GraphPad Prism v9.0.0 and considering a significance level (α) of 0.05.

3. Results

3.1. Environmental data

Intertidal mudflat temperature was highly variable during 2019 and 2020 as expected (Fig. 1). The maximum – MHT (Maximum Habitat Temperature) was registered in summer months (July – September 2019 and 2020), with values ranging between 12.2 and 35.7 °C in 2019 and 12.4 – 37.6 °C in 2020 (Fig. 1).

Salinity data collected in the sampling location showed a mean of 34.9 ± 0.8, a minimum of 32.9 and a maximum of 36.3 during the sampling months. For pH data, the mean value was 8.1 ± 0.4, the minimum was 7.4 and the maximum was 8.7. Generally, these two factors did not vary so much, as opposed to temperature, which is a fairly variable parameter.

3.2. Thermal tolerance limits and physiological traits

Survival percentages from this experiment have already been published (Madeira et al., 2021) and can be found in the supplementary material (Table S4).

Temperature significantly impacted wet weight ($F = 5.0$, $df = 2$, $p = 0.01$) of the ragworms. Salinity ($F = 1.9$, $df = 1$, $p = 0.17$) and temperature × salinity interaction ($F = 0.56$, $df = 2$, $p = 0.57$) did not affect worms' wet weight, except between groups T24S20 and T30S30 where significant differences were reported (Tukey's post-hocs: p value > 0.05) (Fig. S5).

Hediste diversicolor specimens showed CTMax values ranging from 37.3 to 39.1 °C between all treatments. Species intraspecific variability in CTMax was overall low (1.1 %) including within each treatment

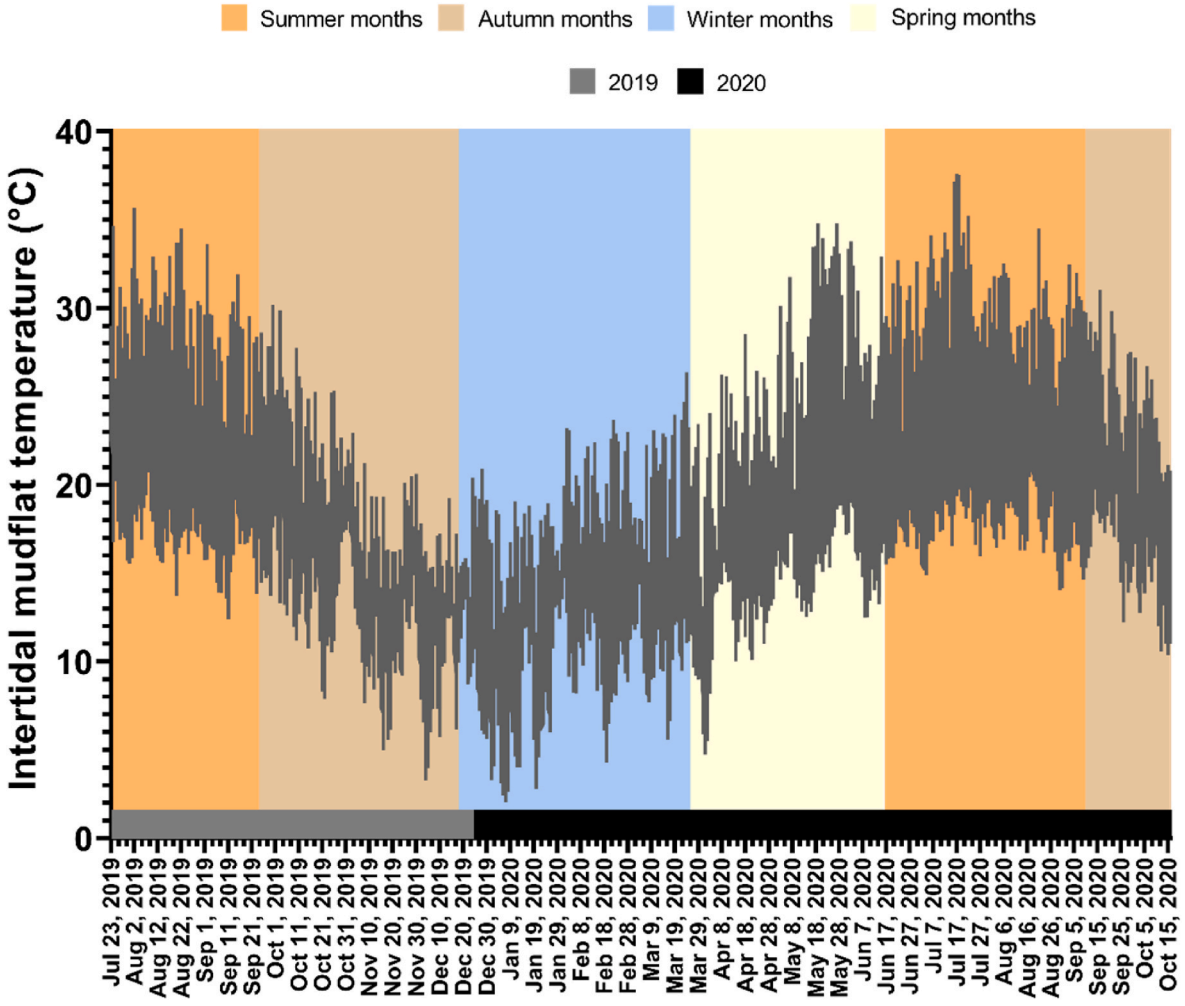

Fig. 1. Temperature measured at the intertidal mudflat where organisms were collected (Ria de Aveiro coastal lagoon, Espinheiro channel, 40°38′3.92″N, 8°39′40.70″W, Aveiro, Portugal) from July 2019 to October 2020. HOBO® data loggers were used (Water Temp Pro v2 U22–001). Seasons are coloured differently: summer (orange), autumn (brown), winter (blue) and spring (light yellow) Adapted from Madeira et al., (2019). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(≤0.85 %) (Fig. 2).

Significant differences in CTMax were observed between acclimation temperatures ($p < 0.0001$) and between salinity values ($p = 0.02$), however no significant interaction was detected between both factors ($p = 0.09$) (Table 1). CTMax values were higher in treatments of higher temperature. Moreover, CTMax values were higher at a salinity of 30, when compared to treatments at a salinity of 20, within the same temperature group (Fig. 2). Thermal Safety Margin (TSM) differed considering the MHT registered between the two years (2019 and 2020) (Table 2). Moreover, in both years, TSM also increased as the acclimation temperature of the treatment was higher (Table 2). Thus, the highest TSM value was detected in the group exposed to 30 °C and a salinity of 30 considering the 2019 MHT (TSM = 2.94 °C). On the opposite, the Acclimation Response Ratio (ARR) values decreased as the acclimation temperature increased (Table 2).

A significant correlation was found between wet weight and CTMax, with a general decrease in CTMax being observed as ragworms weight increased (Fig. 3) (Spearman's correlation, wet weight – CTMax, r = −0.33, $p = 0.03$).

Table 1
Main results of Two-way ANOVA comparing Critical Thermal Maximum (CTMax) values of the marine polychaete *Hediste diversicolor* after 29 days of exposure to several combinations of temperature (T 24, 27 and 30 °C) and salinity (S 20 and 30). Significant results in bold.

Factors	df	F	p-value
Temperature	2	56.00	**<0.0001**
Salinity	1	5.267	**0.0235**
Temperature x Salinity	2	2.465	0.0893

Table 2
Acclimation capacity (CTMax treatment - CTMax control) and ARR (Acclimation Response Ratio) of *Hediste diversicolor* tested under different temperature scenarios (+3 and + 6 °C) combined with salinity (20 and 30). Thermal Safety Margin (TSM) for each treatment, where TSM for each year (2019 and 2020) is the difference between the mean CTMax from each treatment and MHT (Maximum Habitat Temperature) registered in each year (2019 and 2020).

	Treatments (n = 21)					
	T24S20	T24S30	T27S20	T27S30	T30S20	T30S30
Acclimation capacity (°C)	–	–	0.25	0.52	0.58	0.81
TSM (°C) 2019	2.17	2.13	2.42	2.65	2.75	2.94
TSM (°C) 2020	0.21	0.17	0.46	0.69	0.79	0.98
ARR	–	–	0.33	0.33	0.13	0.18

4. Discussion

4.1. Environmental data

As expected, the most variable parameter studied in the sampling location was temperature. Summer mean water temperatures in Ria de Aveiro coastal lagoon, are close to 23 – 31 °C (Almeida et al., 2005; Sousa et al., 2017) during high and low tide (respectively), which is in good agreement with our results. However, our highest recorded temperatures were slightly higher (35 – 37 °C), potentially associated to the fact that mudflats remain with almost no water for several hours. In addition, as MHW tend to be more frequent and stronger, additional monitoring of environmental temperature data over the years is required. Intertidal areas and estuaries have large temperature variations due to tidal cycles (O'Dea and Okamura 1999; Harrison and Whitfield 2006; Lillebø et al., 2015). In *situ* data collection in this study, showed that *H. diversicolor* experiences wide temperature variations and, considering that this ragworm inhabits intertidal mudflats (Scaps 2002), this species could be equipped with plastic and adaptive mechanisms that allow it to endure extreme variations in the environment. Moreover, the highest and lowest temperature values recorded per day, suggest that these organisms live in an environment with large daily thermal ranges, i.e., they must acclimatize quickly in a short time frame, which could be a relevant trait for species survival (Kim et al., 2016).

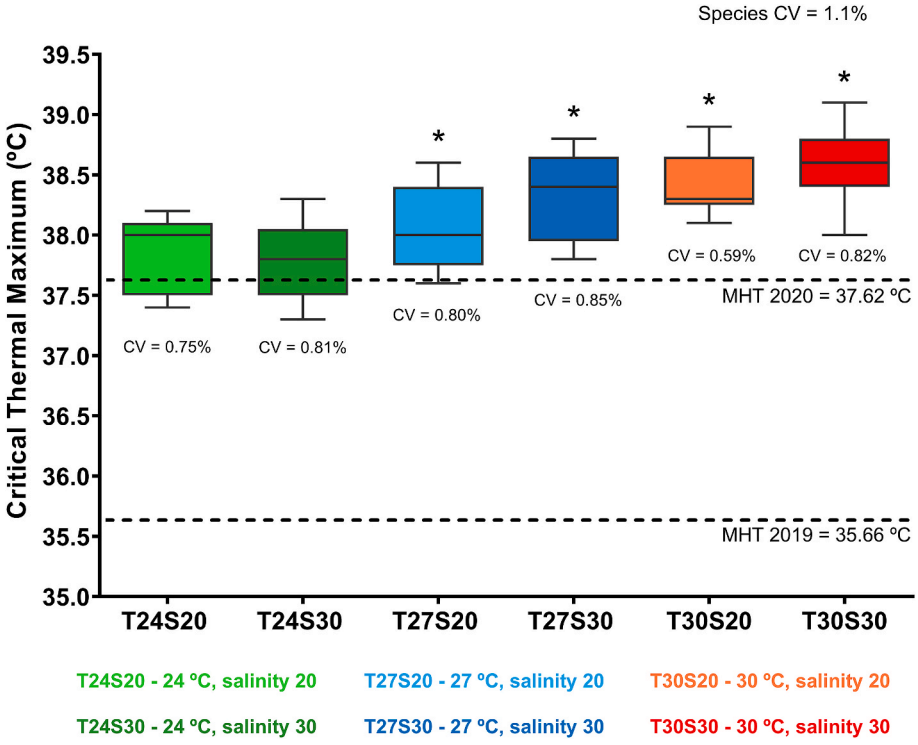

Fig. 2. Box and whiskers plots representing Critical Thermal Maximum (CTMax) values (minimum, maximum, median and quartiles) from each treatment (n = 21). Species % CV (Coefficient of Variation) of all specimens of *Hediste diversicolor* on top (1.1 %) and for each treatment under each box. MHT (Maximum Habitat Temperature) value registered at the sampling location in each year (2019 and 2020 - dashed line). Significant differences (T27S30, T30S20, T30S30 $p < 0.0001$ and T27S20 $p = 0.0239$) when compared to control group (T24S30) are marked with an asterisk (*).

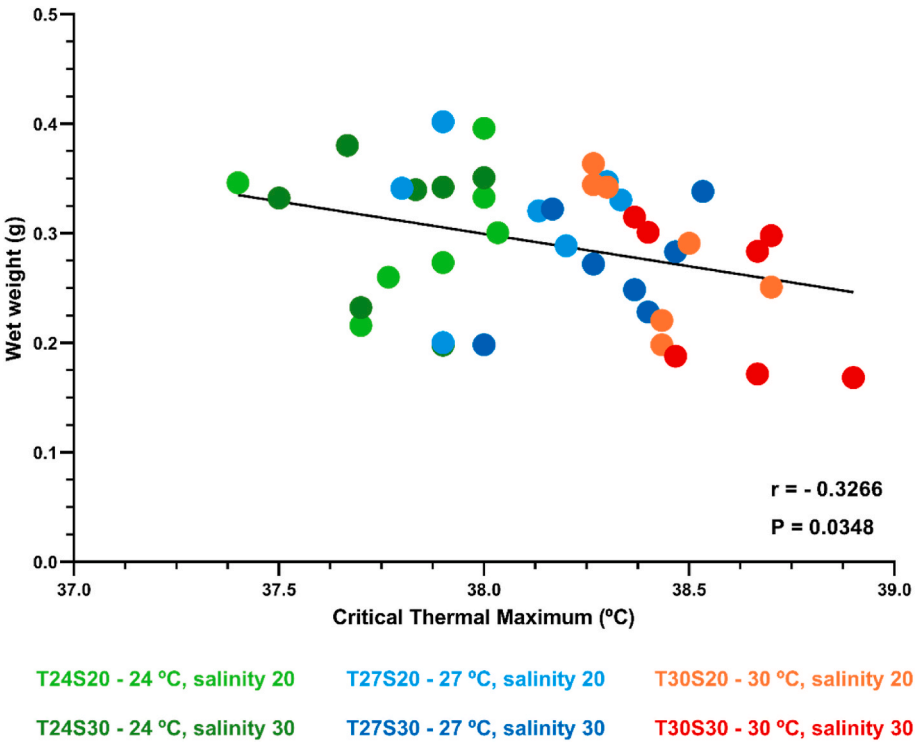

Fig. 3. Correlation between *Hediste diversicolor* wet weight and Critical Thermal Maximum (CTMax) values from each treatment (n = 7 glass flasks per treatment, each with 3 ragworms inside, for which the wet weight and CTMax were averaged).

Salinity showed no considerable variations in the sampling location during the summer, despite being a coastal lagoon environment, where some variation could have been expected (Vaz and Dias 2008). However, major salinity variations are more likely to occur between seasons, due to variations in temperature and rainfall (Lillebø et al., 2015). Within season data are actually in agreement with previous studies in Ria de Aveiro lagoon (Lillebø et al., 2015). During the summer, low freshwater inflow is expected, leading to saltwater propagation further upstream. As a result, the main lagoon channels are largely filled with high saline water, whereas the extreme ends of the lagoon are observed to have oceanic saline water (Vaz and Dias 2008). Some of these channels can reach salinity values over 40, in the summer, at low tide (Sousa et al., 2017). Since this factor does not vary so much in this channel in the summer, presumably, in the winter with rainfall, salinity may have larger variations. Thus, species from this specific location are likely to have mechanisms that allow them to acclimatize to salinity fluctuations.

4.2. *Species upper thermal limits, intraspecific variation and acclimation capacity*

Organisms that were acclimated to higher temperatures had a higher CTMax value than organisms acclimated to lower temperatures. Higher tolerance for organisms that were acclimated to higher temperatures has also been found in other ectotherms, such as fish and shrimps (Stillman 2003; Chown et al., 2009; Peck et al., 2014; Rodríguez-Fuentes et al., 2017; Madeira et al., 2019). *Hediste diversicolor* was capable of shifting CTMax by a maximum of 0.81 °C, following a change of +6 °C in environmental temperature for 1 month. Such shift in CTMax is comparable to values reported in other invertebrate species, such as the intertidal crab *Pachygrapsus marmoratus* (~1 °C, Vinagre et al. (2016), but lower than reported in the intertidal shrimp *Palaemon elegans* (2.8 °C, Vinagre et al. (2016), and intertidal fish *Coryphoblennius galerita* (4.0 °C, Vinagre et al. (2016)). Acute heat shock has also been shown to increase CTMax in the order of 0.83 – 2.42 °C in several marine crustaceans, depending on species and season (Hopkin et al., 2006). These results confirm that acclimation temperature (i.e. recent thermal history) affects thermal tolerance plasticity, thus helping organisms to be more tolerant to a sudden change in water temperature (Galvez 2018; Fox et al., 2019). However, this may not be a general pattern, as decreases in CTMax after heat stress have been reported as well (Hopkin et al., 2006; Vinagre et al., 2018). Nevertheless, overall positive shifts in CTMax should increase population survival, at least in the short-term (Kellermann and Sgrò 2018).

Accordingly, phenotypic plasticity in physiological and life-history traits, occurring within- and trans-generationally, is known to buffer the negative effects of climate change (Gibbin et al., 2017). If adaptive, it allows species to persist in a specific habitat/environment by having a positive effect on fitness (Reed et al., 2011; Grenier et al., 2016; Bonamour et al., 2019) Thus, as suggested by Massamba-N'Siala et al. (2014) and Chakravarti et al. (2016), this improved response capacity may facilitate the ability of populations and species to cope with the challenges raised by climate change within and across generations. However, phenotypic plasticity is a trait that is subject to evolutionary change itself (Gibbin et al., 2017). For instance, phenotypic plasticity should be selected for when the environment shows sustained space and/or time heterogeneity, leading to different phenotypes in different environments (Reed et al., 2011; Chevin and Hoffmann 2017; Brahim et al., 2019). Nonetheless, global change may influence the evolutionary trajectories of phenotypic plasticity by altering the reliability, perception and interpretation of environmental cues, associated with climate stochasticity (Bonamour et al., 2019). Moreover, (i) the production cost of plasticity and (ii) genetic and physiological constraints leading to a saturation state of plastic phenotypic responses, may also limit population persistence under extreme conditions (Chevin et al., 2010). In fact, several studies report that intertidal/eurythermal organisms, despite having high thermal tolerances, have low acclimation capacities as they are already living on the edge of their physiological limits (Stillman 2003; Hopkin et al., 2006). At higher temperatures, plasticity of CTMax might be constrained (as shown by the decrease in ARR with an increase in temperature), suggesting that a trade-off exists between

high thermal tolerance and phenotypic plasticity, as already shown in studies addressing marine invertebrates (Vinagre et al., 2018). Thus, some authors suggest that plasticity in CTMax is somewhat beneficial but may not be enough decrease overheating risk under global change scenarios (Gunderson and Stillman 2015; van Heerwaarden et al., 2016; Barria and Bacigalupe 2017; Gunderson et al., 2017).

Curiously, the CTMax increased with rising salinity, suggesting that the ragworm specimens may prefer a higher salinity (30) over a lower salinity (20). Such result matches well with the salinity measurements carried out in the sampling location, that were always above 30. It is therefore legitimate to assume that a small variation in salinity can be critical under extreme weather events (e.g., MHW), allowing, or not, the species' survival by shifting their thermal tolerance (i.e., CTMax). Additionally, this correlates fairly well with Freitas et al. (2015) and further supports the idea of optimum salinity around 30 for this ragworm species, similarly to other sympatric polychaetes such as *Diopatra neapolitana* (optimum salinity ranging from 28 to 35). If living within their optimum salinity, polychaetes may have an enhanced ability to cope with other stressors, as shown in *D. neapolitana* under these conditions, as it was able to regenerate tissue after injury (Freitas et al., 2015). While there was no significant interaction between temperature and salinity on CTMax values, suggesting that the pattern of response across the levels of one factor is the same at all levels of the other factor (e.g., CTMax increases as temperature rises, at both salinities tested). In this way, *H. diversicolor* may have some ability to cope with predicted extreme events, but further studies are necessary to reach a more robust conclusion.

Overall, in our study, species intraspecific variability in thermal tolerance was low, including within each treatment, suggesting that individuals have a very similar reaction and behavior to thermal and saline stress, even under different conditions. Similar results were obtained for thermal tolerance measurements in crustaceans and fish, in which the CV varied between 0 and 5% (Madeira et al., 2012; Vinagre et al., 2016). Interestingly, intraspecific variation may be greater if populations from different geographic regions are compared, suggesting that heat tolerance should be assessed over the full geographical range of species, as only like this one can accurately estimate climate change impacts on biodiversity (Shaw et al., 2016; Barria and Bacigalupe 2017; Bennett et al., 2019; Herrando-Pérez et al., 2019). Accordingly, organisms can undergo local adaptation or acclimation to the thermal regime on a regional scale (i.e., conspecifics in different geographic locations can have different responses under different environmental stressors) (Freitas et al., 2010), suggesting that populations may differ in climate susceptibility. Other thermal performance related traits also show intraspecific variation, for example aerobic performance in elasmobranchs (Di Santo 2016) and net calcification rates in corals (Shaw et al., 2016), suggesting that intraspecific genetic variability within a population may play an important role in determining adaptive capacity to future conditions.

The significant correlation between wet weight – proxy for body mass - and CTMax is in agreement with previous studies (Selong et al., 2001; Ashaf-Ud-Doulah et al., 2020). Organisms with lower weight had a higher CTMax, suggesting that larger body mass constrains thermal tolerance. Also, this finding may be indicative of trade-offs between thermal limits and growth, potentially associated with energy use for different activities (cellular repair activities vs growth) (Leiva et al., 2019; Kültz 2020). These trade-offs can impact offspring, as the survival of smaller-bodied organisms will be favoured, which can lead to changes in key traits, such as size-at maturity (Gibbin et al., 2017; Thibault et al., 2020) or even halt the species reproduction until an ideal size is attained (Sarà et al., 2014).

The increase of acclimation capacity and TSM as the temperature of experimental treatments was higher, highlighted the ability of ragworms to acclimate to new conditions and increase their TSM, by increasing their CTMax; this feature allowed them to have a larger thermal window to survive extreme weather events, in line with previous studies (Vinagre et al., 2016). However, even though the MHT registered for the two years surveyed *in situ* is lower than CTMax values, an interannual variation of ~2 °C for MHT was recorded. If ocean warming and MHW aggravate, as predicted by the IPCC (IPCC, 2022), it is likely that some species may no longer be able to acclimatize, as they already live very close to their thermal limits. On the other hand, this positive TSM suggests that *H. diversicolor* may have an advantage over other organisms, since in other species TSM can be negative, showing that some of them are already under pressure at present-day temperatures (Madeira et al., 2017). The acclimation response ratio decreased as acclimation temperature was higher, proving that ragworms display some thermal plasticity, but it did not further increase when specimens were acclimated to higher temperatures. This constraint is related with plasticity often decreasing at relatively high acclimation temperatures, by reaching a plateau as shown in other ectotherms (Otto 1973; Fangue et al., 2006; Gunderson and Stillman 2015).

Fluctuations in each single environmental variable may impose significant stress on inhabiting organisms, but the combined exposure to several interacting variables may produce additive and synergistic effects (Côté et al., 2016). Even when animals are adapted to tolerate these extreme environments and populations can persist, the typical environmental variability of estuaries is potentially stressful to animals inhabiting these habitats. More species, different populations and life-stages, as well as multiple generations, need to be tested to confirm these findings, as acclimation capacity can vary among and within taxa, across time and space. Future studies on heat tolerance and phenotypic plasticity should also consider complex ecosystem features, such as species' interactions, community structure and ecosystem function, to allow a more comprehensive assessments of vulnerability to global climate change and marine heatwaves.

Author contributions

DM and RC conceptualization; RC and DM funding acquisition and resources; DM and RC supervision; DM, RC and DJ methodology; JFF, DJ, DM investigation; JFF formal analysis and visualization; JFF writing - original draft; JF, DM, RC and DJ writing – review and editing.

Data availability statement

Physiological dataset is published on Zenodo (https://doi.org/10.5281/zenodo.7795947).

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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.

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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.2023.103577.

References

Abrantes, A., Pinto, F., Moreira, M.H., 1999. Ecology of the polychaete *Nereis diversicolor* in the Canal de Mira (Ria de Aveiro, Portugal): population dynamics, production and oogenic cycle. Acta Oecol. 20, 267–283. https://doi.org/10.1016/s1146-609x(99)00139-3.

Almeida, M.A., Cunha, M.A., Alcântara, F., 2005. Relationship of bacterioplankton production with primary production and respiration in a shallow estuarine system (Ria de Aveiro, NW Portugal). Microbiol. Res. 160, 315–328.

Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford Scholarship, Oxford.

Angilletta, M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. 27, 249–268.

Ashaf-Ud-Doulah, M., Mamun, A.A., Rahman, M.L., Islam, S.M.M., Jannat, R., Hossain, M.A.R., et al., 2020. High temperature acclimation alters upper thermal limits and growth performance of Indian major carp, rohu, *Labeo rohita* (Hamilton, 1822). J. Therm. Biol. 93, 102738.

Barria, A.M., Bacigalupe, L.D., 2017. Intraspecific geographic variation in thermal limits and acclimatory capacity in a wide distributed endemic frog. J. Therm. Biol. 69, 254–260.

Bennett, N.J., Cisneros-Montemayor, A.M., Blythe, J., Silver, J.J., Singh, G., Andrews, N., et al., 2019. Towards a sustainable and equitable blue economy. Nat. Sustain. 2, 991–993.

Bonamour, S., Chevin, L.M., Charmantier, A., Teplitsky, C., 2019. Phenotypic plasticity in response to climate change: the importance of cue variation. Phil. Trans. Biol. Sci. 374, 20180178.

Brahim, A., Mustapha, N., Marshall, D.J., 2019. Non-reversible and reversible heat tolerance plasticity in tropical intertidal animals: responding to habitat temperature heterogeneity. Front. Physiol. 9, 1909. https://doi.org/10.3389/fphys.2018.019099.

Chakravarti, L.J., Jarrold, M.D., Gibbin, E.M., Christen, F., Massamba-N'Siala, G., Blier, P.U., et al., 2016. Can trans-generational experiments be used to enhance species resilience to ocean warming and acidification? Evol. Appl. 9, 1133–1146.

Chevin, L.M., Hoffmann, A.A., 2017. Evolution of phenotypic plasticityin extreme environments. Philosoph. Trans. Royal Soc. B 372, 20160138. https://doi.org/10.1098/rstb.2016.013811.

Chevin, L.M., Lande, R., Mace, G.M., 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biol. 8, e1000357.

Chown, S.L., Jumbam, K.R., Sørensen, J.G., Terblanche, J.S., 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. Funct. Ecol. 23, 133–140.

Claussen, D.L., 1977. Thermal acclimation in ambystomatid salamanders. Comp. Biochem. Physiol. Physiol. 58, 333–340.

Coleman, M.A., Wernberg, T., 2020. The silver lining of extreme events. Trends Ecol. Evol. 35, 1065–1067.

Côté, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their importance in conservation. Proc. Biol. Sci. 283, 20152592.

Di Santo, V., 2016. Intraspecific variation in physiological performance of a benthic elasmobranch challenged by ocean acidification and warming. J. Exp. Biol. 219, 1725.

Fangue, N.A., Hofmeister, M., Schulte, P.M., 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. J. Exp. Biol. 209, 2859–2872.

Fernandes, J.F., Ricardo, F., Jerónimo, D., Santos, A., Domingues, M.R., Calado, R., Madeira, D., 2021. Modulation of fatty acid profiles by global and local ocean change drivers in the ragworm *Hediste diversicolor*: implications for aquaculture production. Aquaculture 542, 736871. https://doi.org/10.1016/j.aquaculture.2021.736871.

Fox, R.J., Donelson, J.M., Schunter, C., Ravasi, T., Gaitán-Espitia, J.D., 2019. Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. Philosoph. Trans. Royal Soc. B 374, 20180174.

Freitas, V., Cardoso, J.F.M.F., Lika, K., Peck, M.A., Campos, J., Kooijman, S.A.L.M., et al., 2010. Temperature tolerance and energetics: a dynamic energy budget-based comparison of North Atlantic marine species. Phil. Trans. Biol. Sci. 365, 3553–3565.

Freitas, R., Pires, A., Velez, C., Almeida, A., Wrona, F.J., Soares, A.M.V.M., et al., 2015. The effects of salinity changes on the Polychaete *Diopatra neapolitana*: impacts on regenerative capacity and biochemical markers. Aquat. Toxicol. 163, 167–176.

Galvez, F., 2018. Physiological and genomic mechanisms of resilience to multiple environmental stressors. In: Burggren, W., Dubansky, B. (Eds.), Development and Environment. Springer International Publishing Cham, pp. 179–201.

Gibbin, E.M., Massamba N'Siala, G., Chakravarti, L.J., Jarrold, M.D., Calosi, P., 2017. The evolution of phenotypic plasticity under global change. Sci. Rep. 7, 17253.

Gillet, P., Torresani, S., 2003. Structure of the population and secondary production of *Hediste diversicolor* (O.F. Müller, 1776), (polychaeta, nereidae) in the loire estuary, atlantic coast, France. Estuar. Coast Shelf Sci. 56, 621–628.

Grenier, S., Barre, P., Litrico, I., 2016. Phenotypic plasticity and selection: nonexclusive mechanisms of adaptation. Scientifica 1–9, 2016.

Gunderson, A.R., Stillman, J.H., 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc. Biol. Sci. 282, 20150401.

Gunderson, A.R., Dillon, M.E., Stillman, J.H., 2017. Estimating the benefits of plasticity in ectotherm heat tolerance under natural thermal variability. Funct. Ecol. 31, 1529–1539. https://doi.org/10.1111/1365-2435.12874.

Harrison, T.D., Whitfield, A.K., 2006. Temperature and salinity as primary determinants influencing the biogeography of fishes in South African estuaries. Estuar. Coast Shelf Sci. 66, 335–345.

Herrando-Pérez, S., Ferri-Yáñez, F., Monasterio, C., et al., 2019. Intraspecific variation in lizard heat tolerance alters estimates of climate impact. J. Anim. Ecol. 88, 247–257. https://doi.org/10.1111/1365-2656.1291429, 2019.

Hofmann, G.E., 2005. Patterns of Hsp gene expression in ectothermic marine organisms on small to large biogeographic scales. Integr. Comp. Biol. 45, 247–255.

Hopkin, R.S., Qari, S., Bowler, K., Hyde, D., Cuculescu, M., 2006. Seasonal thermal tolerance in marine Crustacea. J. Exp. Mar. Biol. Ecol. 331, 74–81.

IPCC, 2019. IPCC Special Report on the Ocean and Cryosphere in a Changing Climate. Cambridge University Press, Cambridge, UK and New York, NY, USA, p. 755. https://doi.org/10.1017/9781009157964.

IPCC, 2022. Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA. https://doi.org/10.1017/9781009157926.

Jackson, M.C., Pawar, S., Woodward, G., 2021. The temporal dynamics of multiple stressor effects: from individuals to ecosystems. Trends Ecol. Evol. 36, 402–410.

Jerónimo, D., Lillebø, A.I., Maciel, E., Domingues, M.R.M., Cremades, J., Calado, R., 2021. Unravelling the fatty acid profiles of different polychaete species cultured under integrated multi-trophic aquaculture (IMTA). Sci. Rep. 11, 10812.

Kaspari, M., Clay, N.A., Lucas, J., Yanoviak, S.P., Kay, A., 2015. Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. Global Change Biol. 21, 1092–1102.

Kellermann, V., Sgrò, C.M., 2018. Evidence for lower plasticity in CT_{MAX} at warmer developmental temperatures. J. Evol. Biol. 31, 1300–1312.

Kim, J.K., Yarish, C., Pereira, R., 2016. Tolerances to hypo-osmotic and temperature stresses in native and invasive species of *Gracilaria* (Rhodophyta). Phycologia 55, 257–264.

Kültz, D., 2020. Evolution of cellular stress response mechanisms. J. Exp. Zool. Part A, Ecological and integrative physiology 333, 359–378.

Laufkötter, C., Zscheischler, J., Frölicher, T.L., 2020. High-impact marine heatwaves attributable to human-induced global warming. Science 369, 1621–1625.

Leiva, F.P., Calosi, P., Verberk, W.C.E.P., 2019. Scaling of thermal tolerance withbody mass and genome size in ectotherms:a comparison between water- andair-breathers. Philosoph. Trans. Royal Soc. B 374, 20190035. https://doi.org/10.1098/rstb.2019.003541.

Lillebø, A.I., Ameixa, O., Sousa, L., Sousa, A.I., Soares, J., Dolbeth, M., et al., 2015. The physio-geographical background and the ecology of Ria de Aveiro. In: Lillebø, A.I., Stalnacke, P., Gooch, G.D. (Eds.), Coastal Lagoons in Europe: Integrated Water Resource Strategies. IWA Publications London, UK, pp. 21–38.

Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: history and critique. Can. J. Zool. 75, 1561–1574.

Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C., 2012. Thermal tolerance and potential impacts of climate change on coastal and estuarine organisms. J. Sea Res. 70, 32–41.

Madeira, C., Mendonça, V., Leal, M.C., Flores, A.A.V., Cabral, H.N., Diniz, M.S., et al., 2017. Thermal stress, thermal safety margins and acclimation capacity in tropical shallow waters—an experimental approach testing multiple end-points in two common fish. Ecol. Indicat. 81, 146–158.

Madeira, C., Mendonça, V., Leal, M.C., Diniz, M.S., Cabral, H.N., Flores, A.A.V., et al., 2019. Present and future invasion perspectives of an alien shrimp in South Atlantic coastal waters: an experimental assessment of functional biomarkers and thermal tolerance. Biol. Invasions 21, 1567–1584.

Madeira, D., Fernandes, J.F., Jerónimo, D., Martins, P., Ricardo, F., Santos, A., et al., 2021. Salinity shapes the stress responses and energy reserves of marine polychaetes exposed to warming: from molecular to functional phenotypes. Sci. Total Environ. 795, 148634.

Marques, B., Lillebø, A.I., Ricardo, F., Nunes, C., Coimbra, M.A., Calado, R., 2018. Adding value to ragworms (*Hediste diversicolor*) through the bioremediation of a super-intensive marine fish farm. Aquacult. Environ. Interac. 10, 79–88.

Massamba-N'Siala, G., Calosi, P., Bilton, D.T., Prevedelli, D., Simonini, R., 2012. Life-history and thermal tolerance traits display different thermal plasticities and relationships with temperature in the marine polychaete *Ophryotrocha labronica* La Greca and Bacci (Dorvilleidae). J. Exp. Mar. Biol. Ecol. 438, 109–117.

Massamba-N'Siala, G., Prevedelli, D., Simonini, R., 2014. Trans-generational plasticity in physiological thermal tolerance is modulated by maternal pre-reproductive environment in the polychaete *Ophryotrocha labronica*. J. Exp. Biol. 217, 2004–2012.

Missionário, M., Fernandes, J.F., Travesso, M., Freitas, E., Calado, R., Madeira, D., 2022. Sex-specific thermal tolerance limits in the ditch shrimp *Palaemon varians*: eco-evolutionary implications under a warming ocean. J. Therm. Biol. 103, 103151.

Moore, M.P., Martin, R.A., 2019. On the evolution of carry-over effects. J. Anim. Ecol. 88, 1832–1844.

Mora, C., Ospína, A., 2001. Tolerance to high temperatures and potential impact of sea warming on reef fishes of Gorgona Island (tropical eastern Pacific). Mar. Biol. 139, 765–769.

Morgan, R., Finnøen, M.H., Jutfelt, F., 2018. CT_{max} is repeatable and doesn't reduce growth in zebrafish. Sci. Rep. 8, 7099.

Müller, O.F., 1776. Zoologiae Danicae prodromus : seu Animalium Daniae et Norvegiae indigenarum ; characteres, nomina, et synonyma imprimis popularium. Typis Hallageriis, Havniae.

Nesto, N., Simonini, R., Prevedelli, D., Da Ros, L., 2012. Effects of diet and density on growth, survival and gametogenesis of *Hediste diversicolor* (O.F. Müller, 1776) (Nereididae, Polychaeta). Aquaculture 362–363, 1–9.

O'Dea, A., Okamura, B., 1999. Influence of seasonal variation in temperature, salinity and food availability on module size and colony growth of the estuarine bryozoan *Conopeum seurati*. Mar. Biol. 135, 581–588.

Otto, R.G., 1973. Temperature tolerance of the mosquitofish, *Gambmia affinis* (baird and girard). J. Fish. Biol. 5, 575–585.

Peck, L.S., Morley, S.A., Richard, J., Clark, M.S., 2014. Acclimation and thermal tolerance in Antarctic marine ectotherms. J. Exp. Biol. 217, 16–22.

Pörtner, H.O., Bock, C., Mark, F.C., 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. J. Exp. Biol. 220, 2685–2696.

Reed, T.E., Schindler, D.E., Waples, R.S., 2011. Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. Conserv. Biol. 25, 56–63.

Rodríguez-Fuentes, G., Murúa-Castillo, M., Díaz, F., Rosas, C., Caamal-Monsreal, C., Sánchez, A., et al., 2017. Ecophysiological biomarkers defining the thermal biology of the Caribbean lobster *Panulirus argus*. Ecol. Indicat. 78, 192–204.

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.

Sarà, G., Rinaldi, A., Montalto, V., 2014. Thinking beyond Organism Energy Use: a Trait-Based Bioenergetic Mechanistic Approach for Predictions of Life History Traits in Marine Organisms, vol. 35, pp. 506–515.

Scaps, P., 2002. A review of the biology, ecology and potential use of the common ragworm *Hediste diversicolor* (O.F. Müller) (Annelida: polychaeta). Hydrobiologia 470, 203–218.

Selong, J.H., McMahon, T.E., Zale, A.V., Barrows, F.T., 2001. Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance in fishes. Trans. Am. Fish. Soc. 130, 1026–1037.

Shaw, E.C., Carpenter, R.C., Lantz, C.A., Edmunds, P.J., 2016. Intraspecific variability in the response to ocean warming and acidification in the scleractinian coral *Acropora pulchra*. Mar. Biol. 163, 210.

Slotsbo, S., Schou, M.F., Kristensen, T.N., Loeschcke, V., Sørensen, J.G., 2016. Reversibility of developmental heat and cold plasticity is asymmetric and has long-lasting consequences for adult thermal tolerance. J. Exp. Biol. 219, 2726–2732.

Somero, G.N., 2011. The physiology of global change: linking patterns to mechanisms. Ann. Rev. Mar. Sci 4, 39–61.

Sousa, A.I., Calado, R., Cleary, D.F.R., Nunes, C., Coimbra, M.A., Seròdio, J., et al., 2017. Effect of spatio-temporal shifts in salinity combined with other environmental variables on the ecological processes provided by *Zostera noltei* meadows. Sci. Rep. 7, 1336.

Spanopoulos-Hernández, M., Martínez-Palacios, C.A., Vanegas-Pérez, R.C., Rosas, C., Ross, L.G., 2005. The combined effects of salinity and temperature on the oxygen consumption of juvenile shrimps *Litopenaeus stylirostris* (Stimpson, 1874). Aquaculture 244, 341–348.

Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. Science 301, 65.

Sunday, J., Bennett, J.M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A.L., et al., 2019. Thermal tolerance patterns across latitude and elevation. Phil. Trans. Biol. Sci. 374, 20190036.

Tewksbury, J.J., Huey, R.B., Deutsch, C.A., 2008. Putting the heat on tropical animals. Science 320, 1296–1297.

Thibault, C., Massamba-N'Siala, G., Noisette, F., Vermandele, F., Babin, M., Calosi, P., 2020. Within- and trans-generational responses to combined global changes are highly divergent in two congeneric species of marine annelids. Mar. Biol. 167, 41.

van Heerwaarden, B., Kellermann, V., Sgrò, C.M., 2016. Limited scope for plasticity to increase upper thermal limits. Funct. Ecol. 30, 1947–1956.

Vaz, N., Dias, J.M., 2008. Hydrographic characterization of an estuarine tidal channel. J. Mar. Syst. 70, 168–181.

Velasco-Blanco, G., Re, A.D., Díaz, F., Ibarra-Castro, L., la Parra, M.I.A., Rodríguez-Ibarra, L.E., et al., 2019. Thermal preference, tolerance, and thermal aerobic scope in clownfish *Amphiprion ocellaris* (Cuvier, 1830) predict its aquaculture potential across tropical regions. Int. Aquat. Res. 11, 187–197.

Vinagre, C., Leal, I., Mendonça, V., Madeira, D., Narciso, L., Diniz, M.S., et al., 2016. Vulnerability to climate warming and acclimation capacity of tropical and temperate coastal organisms. Ecol. Indicat. 62, 317–327.

Vinagre, C., Mendonça, V., Cereja, R., Abreu-Afonso, F., Dias, M., Mizrahi, D., et al., 2018. Ecological traps in shallow coastal waters-Potential effect of heat-waves in tropical and temperate organisms. PLoS One 13, e0192700.

Wang, H., Seekamp, I., Malzahn, A., Hagemann, A., Carvajal, A.K., Slizyte, R., et al., 2019. Growth and nutritional composition of the polychaete *Hediste diversicolor* (OF Müller, 1776) cultivated on waste from land-based salmon smolt aquaculture. Aquaculture 502, 232–241.