Title

Combined effects of temperature and salinity on the metabolic rate of *Heterocypris salina* (Ostracoda, Cyprididae).

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Abstract

Temperature and salinity are considered critical factors affecting the metabolism of freshwater crustaceans. However, experimental research aimed at assessing their interacting effects on oxygen uptake (as a proxy for metabolic rate) has been mainly focused on marine decapods. Here, for the first time, we have experimentally quantified the respiratory responses of a cypridoidean ostracod, *Heterocypris salina* (Brady, 1868), to different combinations of temperature (10, 15, 20, 25, and 30ºC) and salinity (0.5, 5, 10, and 20 PSU). The study species has a Holarctic distribution and high tolerance to a wide range of environmental conditions. As expected, we found significant positive effects of temperature on the ostracod oxygen consumption rate (49% increase per 5ºC on average). Isolated effects of salinity on oxygen uptake were weak. However, we found a strong, negative interaction between salinity and temperature at their upper range (30ºC and 20 PSU), with a 50% mean decline in oxygen uptake. The maximum oxygen consumption rate recorded was 4.15 μL O_2 h^{-1} mg^{-1} (at 30°C and 10 PSU), and the lowest 0.19 μL O^2 h^{-1} mg^{-1} (at 30°C and 20 PSU). Our results support the role of salinity in limiting the metabolic responses of freshwater ostracods to high temperatures.

Keywords

Crustacea, multiple stressors, osmoregulation, Podocopida, respiration.

Introduction

The metabolic rate of an organism is a fundamental ecological trait (Sibly et al., 2012) involved in the balance between oxygen supply and demands that sets the limits of thermal tolerance (Pörtner 2002; Verberk et al. 2016). The metabolic rate of ectotherms (which represent 99% of species on Earth; Wilson 1992; Atkinson and Sibly 1997) is strongly dependent on environmental temperature (Angilletta 2009), and it is often one of the first individual traits that respond to climate change (Bruno et al. 2015; Verberk et al. 2016). The relationship between temperature and metabolic rate is often considered as unimodal within the limits of functional integrity, with an increasing pattern at moderate temperatures until reaching a critical thermal maximum, before a monotonic fall in the metabolic rate occurs, indicating a mismatch between oxygen availability and demand leading to the collapse of physiological function (Pörtner 2002; Alcaraz et al. 2014). Metabolic responses to temperature strongly depend on body mass (Sibly et al., 2012), and may also be affected by climate adaptations at different latitudes (Shokri et al. 2022; Shokri et al. 2024). Under global warming, the metabolic performance of many aquatic ectotherms will be compromised by an increased oxygen demand that may exceed the available supply (Verberk et al. 2011; Breitburg et al. 2018). The effects of increasing temperature on this oxygen challenge can be alleviated via plastic responses involving a variety of biological mechanisms acting at different temporal scales (Pascal and Chong 2016; Lehette 2017; Rangel and Sorte 2022) and organisation levels (Kielland et al. 2019).

Salinity is another critical factor affecting oxygen consumption rates of aquatic organisms (Del Giorgio and Williams 2005). Crustaceans have developed different molecular, physiological, ecological, and behavioral mechanisms to cope with salinity changes that allow them to inhabit an exceptionally broad range of habitats, spanning from freshwater to brackish and marine waters (Thabet et al. 2017). Physiological responses of crustaceans to salinity have energetic costs that influence metabolic responses. For instance, the high-shore rockpool copepod *Tigriopus brevicornis* experienced a reduction in oxygen consumption rate when exposed to very low salinity, attributed to the cost of osmoregulation (McAllen and Taylor 2001). Individuals of the amphipod *Gammarus colei* showed an active respiratory response to acute osmotic stress until a salinity threshold was reached, above which they collapsed because they lacked the energy required to respond to that stress (Burns et al. 2022); however, in the same study, another amphipod species of the genus *Hyalella* did not show such a response. Contrasting species-specific respiratory responses to salinity were also observed in anchialine shrimps (Havird et al. 2019; Chávez Solís et al. 2024). Furthermore, respiratory response to salinity can vary across developmental stages, as observed in large branchiopods (El-Gamal 2011).

The impacts of global change on biodiversity ultimately depend on physiological responses of individuals to shifts in multiple environmental stressors (Pörtner 2012; Sokolova 2013; Todgham and Stillman 2013; Breitburg et al.

2018; Tripp et al. 2022). Given that both water temperature and salinity dynamics will be affected by global warming, understanding how salinity interacts with temperature in shaping metabolic rates is crucial. This is particularly relevant in shallow coastal wetlands that experience large variability in temperature and salinity, such as those of Mediterranean region (Margalef 1983).

Previous studies investigating the effects of temperature and salinity on oxygen consumption rates of aquatic crustaceans are mainly focused on Decapoda (e.g., Dalla Via 1987; Winch and Hodgson 2007), with other crustacean groups being less commonly treated (but see Gyllenberg and Lundqvist 1979; Varó et al. 1993; Garreta-Lara et al. 2018). Indeed, decapods have been traditionally used as model organisms of crustacean physiology (McNamara and Faria 2012). However, because crustaceans are highly diverse in their respiratory structures (Harrison 2015) and osmoregulatory mechanisms (Henry et al. 2012), respiratory responses of decapods to temperature and salinity might not reflect those of other crustacean groups. This might be the case of podocopid ostracods, a group of small crustaceans with primitive morphofunctional respiratory structures (e.g. small branchial plates in appendage exopods) and without blood circulatory systems (Corbari 2004). Interestingly, preliminary data indicate that ostracods are among the crustaceans with the lowest mass-specific oxygen consumption rates (Ivleva 1980). Nevertheless, only a few previous publications assess thermal and/or salinity effects on ostracod respiration, one focusing on marine planktonic species of the subclass Myodocopa (Ikeda 1990), and other six on benthic podocopids. Four of those dealt with brackish and freshwater ostracods of the podocopid superfamily Cytheroidea (Hagerman 1969; Herman and Heip 1982; Newrkla 1985; Peper 1986), whereas the other two examine non-marine cypridoideans (Ganning 1971; Grant et al. 1983). Importantly, only two previous publications assessed the combined effects of temperature and salinity on ostracod oxygen uptake, both involving cytheroidean ostracods (Hagerman 1969; Peper 1986).

In the present work, we quantified the respiratory responses of a cypridoidean ostracod to different combinations of temperature (10, 15, 20, 25, and 30ºC) and salinity (0.5, 5, 10, and 20 PSU). The study species is *Heterocypris salina* (Brady, 1868), a widely distributed non-marine, halophilic ostracod (Meisch 2000). In a previous study, Ganning (1971) observed a slight increase in oxygen consumption rates of *H. salina* with increasing salinity (from 1 to 20 PSU) for a constant temperature of 15°C. Here, we experimentally accounted for the interactive effects of salinity and temperature on individuals of *H. salina* inhabiting a shallow coastal wetland of the Western Mediterranean region, providing evidence of the role of salinity in constraining the metabolic responses of a non-marine podocopid ostracod to temperature.

Materials and Methods

Study species

The species *Heterocypris salina* (Brady, 1868) is a podocopid ostracod representative of the superfamily Cypridoidea, family Cyprididae, subfamily Cyprinotinae. Podocopid ostracods live mostly in epicontinental, coastal and estuarine aquatic environments, including inland, brackish and marine species, mostly with a benthic, phytophilic, nekto-benthic or hypogeous lifestyle, with reduced swimming abilities in many species (Baltanás and Mesquita-Joanes, 2015). Cypridoidea, particularly those of the family Cyprididae, are relatively scarce in marine habitats but dominant and highly diverse in freshwaters (Meisch, 2000; Martens et al. 2008). Like most members of the Cyprinotinae, the species *H. salina* is able to tolerate desiccation. It is found almost exclusively as asexual populations of parthenogenetic females (with no males), and displays exceptionally high morphological variation and genetic diversity (Kilikowska et al. 2024). It is widely distributed throughout Europe, the Azores, North Africa and West Asia (Meisch 2000; Kilikowska et al. 2024). It can be found in a wide variety of aquatic habitats, including rockpools, springs, ponds, troughs, streams, wetlands and lakes (e.g., Ganning 1971; Mezquita et al. 1999; Altinsaçlı and Mezquita 2005; Valls et al. 2016; Külköylüoğlu et al. 2017; Gusakov et al. 2021)

Collection, maintenance, and selection of experimental specimens

The experimental individuals of *H. salina* were collected in the Fondo d'Elx Natural Park (Latitude: 39º11'9.41"N; Longitude: 0º47'8.87") in two sampling occasions between December 2021 and January 2022, both in the same site. The physicochemistry (temperature, salinity, and pH) of the water was 13-14ºC, 4.5- 5.4 PSU, and 7.8, respectively. Notice that, in this study, we represent salinity in PSU (i.e., practical salinity units), indicating that we estimated salinity from conductivity using a WTW conductimeter. Although they can differ from salinity estimations based on chlorinity, these differences are expected to be very small, generally in the range of 0.001-0.03 PSU (Lewis and Perkin 1981; Pawlowicz 2013) and, therefore, can be neglected in our framework of crustacean metabolic responses to salinity variation. The major ionic composition of the sampling site, according to a previous publication (Rodrigo et al. 2002), is dominated by Cl- and Na+, with an Cl:SO4:CO3 relationship of approximately 50:20:1. The sampled population of *H. salina* was composed of parthenogenetic females (no males were found). The ostracods were collected by filtering water and bottom sediment using hand nets with a mesh size of 250 µm. The sampled ostracods were transported in jars with water from the sampling place, in a portable refrigerator to keep them cool during transport. At the laboratory, ostracods were placed in aquaria with water from their place of origin, at the ambient temperature of the laboratory, and an aerator was installed to maintain the water highly oxygenated. The day before the experiment, ostracods were isolated using

Pasteur pipettes, counted and transferred to new containers with water from their place of origin, previously filtered through a 0.7 μm Whatman® GF/F filter to remove microorganisms. Small spinach fragments were added as food to the containers, following Schmit et al. (2007). In the experiments, we selected only adults (1.12-1.25 mm of length; Ganning 1971) to reduce the effects of body size and age on oxygen consumption rates (Bridges and Brand 1980; El-Gamal 2011).

Respirometry experiments

We performed a series of respirometry experiments at different combinations of temperature (10, 15, 20, 25, and 30ºC) and salinity (0.5, 5, 10, and 20 PSU), with eight replicates and two controls per combination of temperature and salinity. Each replicate consisted of a 2.7 ml vial containing 15 experimental individuals (adult females of *H. salina*) and the water corresponding to the experimental treatment. Controls were vials with the corresponding water treatment but no ostracods.

Previous to the experiments, we prepared the experimental media based on commercial Solán de Cabras® mineral water (ionic composition: 284 mg L^{-1} of bicarbonate, 59.4 mg L^{-1} of calcium, 25.6 mg L^{-1} of magnesium, 21.3 mg L^{-1} of sulphate, 7.4 mg L^{-1} of chloride, 7.2 mg L^{-1} of silica, 5.1 mg L^{-1} of sodium, 1.1 mg L^{-1} of potassium; dry residue: 260 mg L^{-1}; conductivity: 0.48 mS cm^{-1}; salinity: 0.2 PSU). The media for the four salinity treatments (0.5, 5, 10, and 20 PSU) were obtained by adding sea salt for aquaria (SERA®) to the commercial water. The added salts were dissolved with the help of a stirrer and applying heat. Once the salts were dissolved, conductivity and salinity were measured using a conductivity meter (WTW® Cond 330i) and the experimental water was autoclaved and cooled for later use. Because we used marine salt to increase salinity of the mineral water, we expect a ionic composition similarly dominated by chloride and sodium, as the field water of the sampling site.

Oxygen consumption data were obtained using a PreSens® SDR2 optical sensor plate, which measures the fluorescence emitted by a foil at the base of transparent 2.7 mL vials containing the experimental samples. The foil is in contact with the experimental water and contains a ruthenium derivative that emits fluorescence in the presence of oxygen (Köster et al. 2008). The optical sensor plate has 24 channels capable of reading oxygen consumption through the sensors of 24 vials at a time. For each experiment, the sensor plate with the vials was placed within a BINDER® KBW incubator chamber maintained at constant temperature, and with a constant light intensity of 20 μE m^{-2} s^{-1}. The plate was connected to a computer via an RJ45 cable through which oxygen concentration data were recorded every 3 minutes, using the SDR_v4.0.0 software.

Before starting each experiment, the water of the corresponding salinity was filtered and shaken for a few minutes to ensure high enough oxygen concentration at the beginning of the experiment. Experimental vials were then partially filled with the water, and 15 ostracods were introduced in each vial (except for the control vials). Finally, the vials were filled to the top without leaving any bubbles, closed with a tight cap and placed in a multiwell plate attached to the optical sensor plate inside the culture chamber.

For each salinity treatment, respirometry measurements were consecutively taken at all temperatures (from lower to higher temp.), starting at the lowest temperature of 10ºC. At each temperature, an initial period of 1.0-1.5 hours was established until the stabilisation of oxygen concentration measurements in the controls. Oxygen concentration in each vial was then recorded for one hour. Afterwards, the chamber temperature was changed and the stabilisation period of the next treatment started. During the experimental time, we checked that oxygen concentration never fell below 4 mg L^{-1}, to avoid potential negative effects of hypoxia for the ostracods. Once measurements at all temperatures were taken, the state of the ostracods (alive or dead) was checked, and ostracods from each vial were stored in microtubes with ethanol for the estimation of dry weight (see below).

Calculation of mass-specific oxygen consumption rates

For each treatment vial, the oxygen consumption rate was initially calculated as the slope of the relationship between oxygen concentration and experimental time. Data from the stabilisation periods were disregarded. The average value of the slopes from the two control vials (which was close to 0) was subtracted from the slope of the corresponding treatment vial to eliminate the potential basal consumption of micro-organisms that might have remained in the medium. These control-corrected consumption rates were then divided by the estimated dry weight of all the specimens in each vial. These mass-specific oxygen consumption rates for each treatment were calculated in units of μL O_2 h^{-1} mg^{-1}, commonly used in the literature.

The dry weight of the group of individuals from each vial was estimated following the methodology used by Herman and Heip (1982) and the standardized procedure for sediment drying suggested by Heiri et al. (2001). First, we dried empty Elemental Microanalysis® aluminium capsules (5 x 3.5 mm) for one hour at 105ºC using a Memmert® UN55 oven, and then we weighed the capsules with a VWR® SM635i-ION microbalance (resolution 0.01mg) to obtain their average weight based on three measurements. Second, ostracods were introduced into the capsules and oven-dried for 4 hours at 105ºC, and then the capsules with the ostracods were weighed, and their average weight was obtained. Finally, the mean weight of the empty capsules was subtracted from the mean weight of the capsules with ostracods, thus obtaining the average dry weight of all the individuals in each treatment vial.

Statistical analysis

The effects of salinity and temperature on mass-specific oxygen consumption rates were assessed with a generalised linear model of gamma family and a logarithmic link function (Ng and Cribbie 2017). This type of model was used because the response variable was continuous, had a range from 0 to +∞ and a slightly right-skewed distribution. We included temperature, salinity, and their interaction as explanatory variables. Both explanatory variables were treated as categorical, which would allow us to capture potential non-linear patterns in their effects across their range. All statistical analyses were performed using R software v. 4.2.0 (R Core Team, 2022).

Results

The experimental results show an increase in oxygen consumption with temperature across the whole range, except for the highest salinity treatment, where the consumption increases within the temperature range between 10 and 25ºC, but drops at the maximum temperature of the experiment (30ºC) (Figure 1). Furthermore, the consumption rates are similar among all salinity treatments except for the highest salinity of 20 PSU, where we find a sharp decrease in oxygen consumption, especially evident at 30ºC. In general, the higher the temperature, the more respiration, but at high salinity (20 PSU) the respiration decreases. The highest average value of oxygen consumption was recorded at the salinity of 10 PSU at 30°C (4.15 µL O_2 h^{-1} mg^{-1}), while the lowest value was 0.19 µL O_2 h^{-1} mg^{-1} at 20 PSU and 30°C. All individuals were alive after the end of the experiment.

The generalised linear model (Table 1) estimated an average expected consumption for the base treatment (0.5 PSU and 10°C) of 0.71 µL O_2 h^{-1} mg^{-1} (model intercept in Table 1). Temperature shows positive isolated effects over its entire range, and the effect size increases with temperature. A 5-degree increase over the base treatment leads to a 49% increase in oxygen consumption, while at the highest temperature consumption increases more than threefold over the base treatment. In contrast, salinity shows weak isolated effects, but significant interaction effects with temperature. The model confirms the negative interaction between salinity and temperature suggested by the graphical representation of the averages of consumption by treatment (Figure 1). Specifically, the interaction between the highest levels of both salinity and temperature reduces oxygen consumption by 50% compared to the expected consumption based on the isolated effects of the two factors (Table 1).

Discussion

Here, we experimentally assessed the combined effects of temperature and salinity on the oxygen consumption rate of *Heterocypris salina*, a widespread cypridoidean ostracod inhabiting continental waters. We obtained the following three main results: (i) we found strong positive effects of temperature, (ii) the isolated effects of salinity were marginal, and (iii) we evidenced a negative interaction of both factors in their upper range.

Strong effects of temperature

We found strong metabolic responses to temperature in *H. salina*, with average effect sizes ranging from a 49% (at 15ºC) to three-fold increases (at 30ºC) relative to the lowest temperature (10ºC; Table 1). Our results agree with the expected increase of metabolic rate within the limits of functional integrity in ectotherms (Pörtner 2002; Alcaraz et al. 2014). The positive correlation between metabolic rate and temperature was also observed in other ostracods (Hagerman 1969; Herman and Heip 1982; Grant et al. 1983; Newrkla 1985; Peper 1986; Ikeda 1990), and other crustaceans, including Decapoda (e.g., Dalla Via 1987; Modlin and Froelich 1997; Winch and Hodgson 2007), Amphipoda (Dorgelo 1973), Copepoda (McAllen et al. 1999; Ikeda et al. 2001), and Anostraca (Modlin 1983; Hazelwood and Hazelwood 1985). The monotonic relationship between respiration rate and temperature along all the temperature range in most of the experimental salinities, plus the fact that all the experimental individuals were alive at the end of the experiment, indicate that the critical temperature in *H. salina* is above the temperature range selected in our experiments, although reduced respiration is already observed at 30°C for high salinities (20 PSU), which is in correspondence with the higher mortality recorded by Ganning (1971) for this species at that temperature, although using individuals from a population at a much higher latitude.

Marginal, non-linear effects of salinity

A previous study suggested that *H. salina* increases oxygen uptake with salinity (Ganning 1971) for a salinity range from 1 to 20 PSU, and a constant temperature of 15°C. Focusing on this reference temperature, our results based on the same salinity gradient indicate a non-linear relationship, with an increase in oxygen uptake up to 10 PSU (in agreement with Ganning 1971), but followed by a drop at 20 PSU that differs from Ganning's results. In line with our results, a study on cytheroidean ostracods from the Baltic Sea also showed that the effect of salinity on ostracod oxygen consumption rates can be negative at high ranges (i.e., >20 PSU). In particular, the ostracod *Eucytheridea bairdii* (Sars, 1866) reduced its metabolism at 30 PSU by closing the valves and consuming less oxygen (Peper 1986). A similar response was described in the high-shore rockpool copepod *Tigriopus brevicornis*. When exposed to an extremely high

244 salinity medium (> 80 PSU), the copepod entered a quiescent state with a large reduction in activity and oxygen
245 consumption (McAllen and Taylor 2001). Some gammarid species also experienced reversible reduction of oxygen
246 consumption under high-salinity stress (Dorgelo 1973). Considering the full range of experimental temperatures, we did
247 not find evidence for a consistent positive correlation of oxygen consumption with salinity at any of the other
248 temperature treatments tested in our study (10, 20, 25, and 30°C). Neither we found the typical increase of metabolic
249 rate with decreasing salinity observed in marine crustaceans (Taylor 1977; McAllen and Taylor 2001) and attributed to
250 the costs of osmoregulation (Mantel and Farmer 1983). Indeed, our model results suggest that, overall, the main effects
251 of salinity on oxygen consumption are weak in *H. salina*. The average sizes of isolated effects of salinity were more
252 moderate than those of temperature, never exceeding a 30% increase or decrease with respect to the base treatment
253 (10°C and 0.5 PSU), and showed either marginal or no significance. Hagerman (1969) also found weak effects of
254 salinity on the oxygen uptake of the cytheroidean ostracod *Hirschmannia viridis* at 10°C. He found a non-linear pattern
255 at 20°C, with a minimum consumption rate at 15 PSU, coinciding with the salinity of the sampling area (Hagerman
256 1969). By contrast, our data on the cypridoidean *H. salina* at 20°C shows a minimum consumption rate at a lower
257 salinity level (i.e., 5 PSU). Accordingly, Ganning (1971) determined an optimal salinity for survival of *H. salina* at 5 to
258 10 g L^{-1}, and Gusakov et al. (2021) reported *Heterocypris salina* to be dominant in mesohaline rivers, but absent from
259 polyhaline ones, i.e. with a preference for salt contents around 5-18 PSU, and not tolerating higher values. Interestingly,
260 most cytheroidean ostracods are marine, with relatively few species inhabiting freshwater habitats, whereas
261 cypridoidean ostracods dominate continental waters (Meisch 2000). Our results highlight the ability of cypridoideans to
262 better perform in water bodies with relatively low salt contents, although we should also test some highly halotolerant
263 cypridoideans, such as those living in hypersaline playa lakes (Baltanás et al. 1990).
264
265 *Negative effects of temperature-salinity interaction*
266 Our experimental results indicate that salinity modulates the metabolic response of *H. salina* to temperature. Focusing
267 on the upper thermal range (25-30°C), we observed a qualitative shift in the effects of temperature on ostracod oxygen
268 uptake driven by salinity, from positive (0.5-10 PSU) to negative (20 PSU). Only two previous publications assess the
269 combined effects of temperature and salt content on ostracod oxygen uptake, both involving cytheroidean ostracods
270 (Hagerman 1969; Peper 1986). Peper (1986) focused on marine species from the Baltic Sea and did not confirm a clear
271 interaction between both abiotic variables. Interestingly, Hagermann (1969) observed a temperature-salinity interaction
272 in the brackish-water ostracod *H. viridis*, with a pattern similar to ours (see Figs 5 and 6 in Hagermann 1969). The
273 interactive effects of temperature and salinity have been documented in other crustaceans, including decapods (Winch

and Hodgson 2007) and amphipods (Dorgelo 1973). For instance, the combination of high temperature and salinity reduces oxygen consumption rates in large individuals of the decapod *Cyclograpsus punctatus* (Winch and Hodgson 2007).

Future perspectives

Considering the three different ostracod lineages that have independently invaded non-marine habitats (i.e., Darwinuloidea, Cypridoidea, and Cytheroidea; Martens 1998), Cypridoidea is, by large, the one with the majority of current freshwater forms, representing c.a. 75.5% of the ~2300 extant non-marine ostracod species (Meisch et al. 2024). The superfamily includes species with habitat preferences ranging from hypo- to hypersaline waters. The replication of our experiments on other cypridoideans with different salinity preferences than *H. salina* would allow to test for interspecific variation in the response of freshwater ostracods to environmental change. For instance, *Eucypris virens* and *Cyclocypris ovum* are potential model candidates for testing ostracod adaptation to low salt content (Meish 2000; Schmit et al. 2013). By contrast, *Arctocypris mareotica* and *Heterocypris barbara*, species found in hypersaline lakes (Baltanás et al. 1990), would represent the other end of the salinity spectrum. It would be also interesting to check whether or not there are differences in metabolic response to salinity and temperature among the three non-marine ostracod lineages, given their contrasting levels of diversification in freshwaters.

Beyond the total salt content, many autoecological studies suggest that the specific hydrochemical composition of waters is also relevant for the distribution of small aquatic organisms (Wetzel 2001), such as diatoms (Gasse et al. 1995), rotifers (Miracle et al. 1987), and ostracods (e.g., Smith 1993; Van der Meeren et al. 2010). An interesting step further would be to investigate the effects of major ion composition on ostracod respiratory physiology, for instance by comparing respiratory performance under water types with similar salinity contents but different ionic composition (e.g., calcium-bicarbonate vs. sodium-chloride dominated types).

Respiration rates are affected by activity levels (e.g., Svetlichny and Hubareva 2005). In our study, we did not measure the degree of locomotory activity of experimental individuals, and assumed all individuals had similar activity levels. The study species, *H. salina*, is a swimmer. Another promising research would be to compare its respiratory performance with that of other ostracods that are crawlers, such as *Herpetocypris reptans*, and using anaesthetics for measurements of basal respiratory rates. With such an approach, we could evaluate the physiological costs of different types of locomotion and their implications in the ostracod responses to climate change.

Overall, our findings line up with other research stressing the importance of considering the interactions between multiple environmental stressors when assessing the impacts of climate change on biodiversity (Pörtner 2012; Sokolova 2013; Todgham and Stillman 2013; Breitburg et al. 2018; Tripp et al. 2022). Our study confirms that physiological responses of freshwater crustaceans to future scenarios of global warming cannot be properly predicted without accounting for the role of salinity levels in shaping these responses.

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Disclosure statement

The authors report there are no competing interests to declare.

Declaration of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

The experimental dataset is available from Zenodo Repository: https://doi.org/10.5281/zenodo.16087765.

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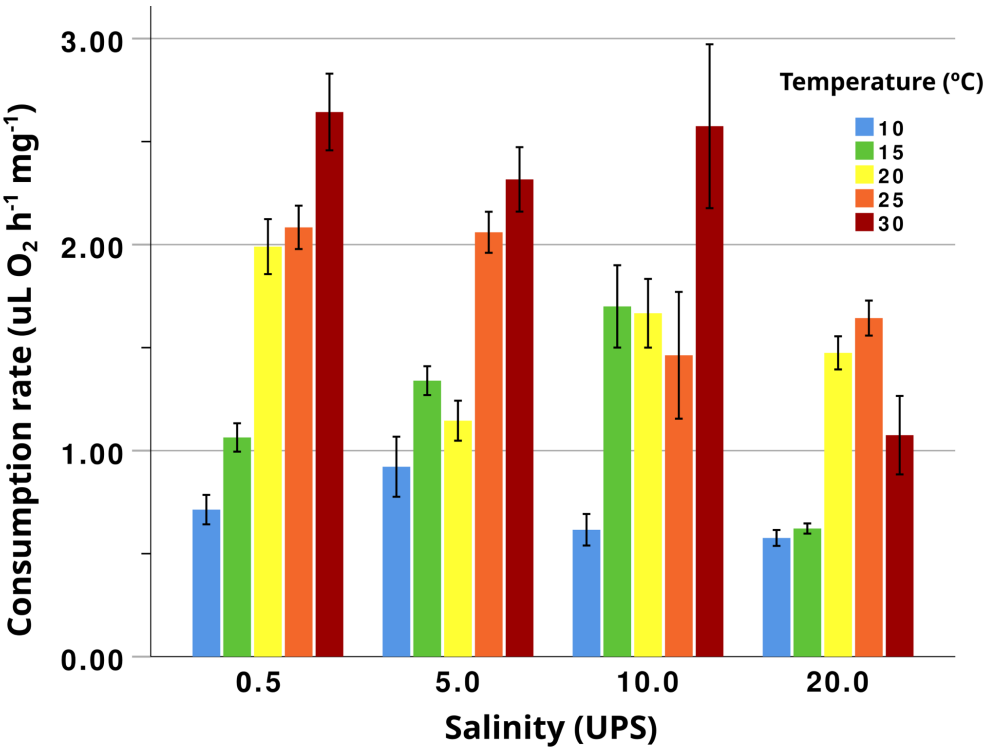
Tables

Table 1. Summary of the Generalized Linear Model used to test the effects of temperature (10, 15, 20, 25, and 30ºC) and salinity (0.5, 5, 10, and 20 PSU). and their interactions, on oxygen consumption rates of *H. salina*. The table shows the name of effect (S = salinity; T = temperature), the estimate of the average effect size expressed as Relative Risk (exponentiated β values), their 95% confidence intervals (CI95), the test statistic (t-value), and the P-value, with significance levels of 0.05(*), 0.005(**), and 0.0005(***). The exponentiated β values (RR) are interpreted as ratios: RR = 1 means no effect; RR > 1 means positive effect; RR < 1 means negative effect. The reference levels of the model, represented by the intercept, were S0.5 and T10.

Effect	Effect size (RR)	CI95	t-value	P-value
Intercept	0.71	[0.60, 0.86]	-3.69	0.0003***
S5	1.29	[1.00, 1.67]	1.98	0.0499*
S10	0.86	[0.64, 1.18]	-0.93	0.35
S20	0.80	[0.63, 1.04]	-1.66	0.10
T15	1.49	[1.16, 1.92]	3.10	0.0025**
T20	2.79	[2.15, 3.63]	7.67	<0.0001***
T25	2.92	[2.25, 3.80]	8.01	<0.0001***
T30	3.70	[2.85, 4.82]	9.79	<0.0001***
S10:T15	1.85	[1.21, 2.83]	2.85	0.0052*
S5:T20	0.45	[0.31, 0.64]	-4.35	<0.0001***
S5:T30	0.68	[0.47, 0.98]	-2.09	0.039*
S20:T30	0.50	[0.35, 0.73]	-3.69	0.0003***

Figure captions

Figure 1. Variation of oxygen consumption rate of *H. salina* depending on temperature and salinity. Coloured bars indicate average values, and error bars represent standard errors.



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