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Carbonell, JA

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Metabolic and reproductive plasticity of core and marginal populations of the eurythermic saline water bug *Sigara selecta* (Hemiptera: Corixidae) in a climate change context.

AUTHORS: J.A. Carbonell[1], D.T. Bilton[2], P. Calosi[3], A. Millán[1], A. Stewart[4]& J. Velasco[1]

1. Departamento de Ecología e Hidrología, Facultad de Biología, Campus de Espinardo, 30100, Universidad de Murcia. Murcia, Spain.
2. Marine Biology and Ecology Research Centre, School of Marine Science and Engineering, University of Plymouth, Davy Building, Drake Circus, Plymouth PL4 8AA, UK.
3. Département de Biologie Chimie et Géographie, Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski, Québec, G5L 3A1, Canada.
4. School of Life Sciences, University of Sussex, Falmer, Brighton, Sussex, BN1 9QG, UK.

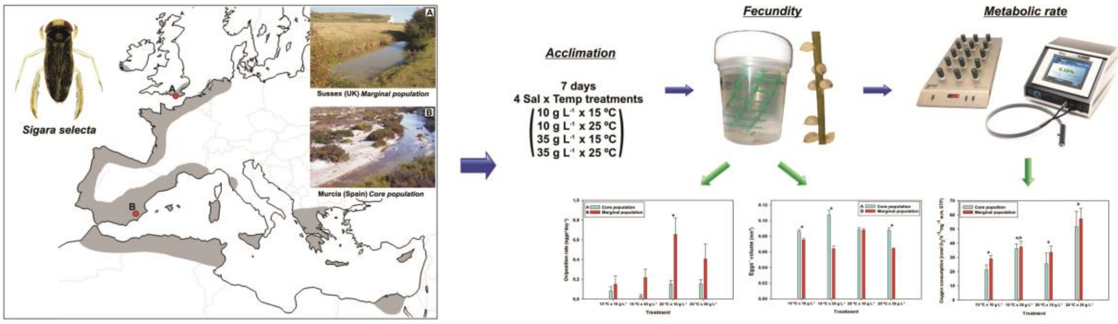
Corresponding author: joseantonio.carbonell@um.es

ABSTRACT

Ongoing climate change is driving dramatic range shifts in diverse taxa worldwide, and species responses to global change are likely to be determined largely by population responses at geographical range margins. Here we investigate the metabolic and reproductive plasticity in response to water temperature and salinity variation of two populations of the eurythermic saline water bug *Sigara selecta*: one population located close to the northern edge of its distribution, in a relatively cold, thermally stable region (SE England – 'marginal'), and one close to the range centre, in a warmer and more thermally variable Mediterranean climate (SE Spain – 'core'). We compared metabolic and oviposition rates and egg size, following exposure to one of four different combinations of temperature (15 and 25 ºC) and salinity (10 and 35 g L^{-1}). Oviposition rate was significantly higher in the marginal population, although eggs laid were smaller overall. No significant differences in oxygen consumption rates were found between core and marginal populations, although the marginal population showed higher levels of plasticity in both metabolic and reproductive traits. Our results suggest that population-specific responses to environmental change are complex and may be mediated by differences in phenotypic plasticity. In *S. selecta*, the higher plasticity of the marginal population may facilitate both its persistence in current habitats and northward expansion with future climatic warming. The less plastic core population may be able to buffer current environmental variability with minor changes in metabolism and fecundity, but could be prone to extinction if temperature and salinity changes exceed physiological tolerance limits in the future.

KEYWORDS: Ecophysiology, range margins, metabolic rate, fecundity, trade-offs, global change.

GRAPHICAL ABSTRACT



HIGHLIGHTS

- Metabolic and reproductive traits of core *vs* marginal populations of *Sigara selecta*.
- Population responses to climate change are mediated by phenotypic plasticity.
- Marginal population shows higher metabolic and fecundity plasticity.
- Marginal population may increase its fitness and expand its range northwards in the future.
- Core population could be more prone to extinction with climate warming.

1. INTRODUCTION

The vulnerability of a species to global warming largely depends on its capacity to maintain present populations (species persistence) and to shift its geographical range to suitable future environments (Parmesan, 2006; Williams et al., 2008), factors heavily influenced by population responses at range margins (Kirkpatrick and Barton, 1997; Hampe and Petit, 2005; Gaston, 2009). The persistence of populations will depend on their ability to adapt to and tolerate novel conditions, i.e. on physiological tolerance limits, as well as the potential to shift these limits *via* plastic and adaptive changes (Chown et al., 2007; Gaston et al., 2009; Hoffmann, 2010).

The adaptive ability of marginal populations can be limited because they are typically less diverse genetically than those close to the range centre (the "centre-periphery hypothesis" of Mayr, 1963), since they tend to occur in less favorable habitats at lower and more variable densities, and often experience stronger genetic drift (Lawton, 1993; Vucetich and Waite, 2003; Hampe and Petit, 2005). However, phenotypic plasticity, or the capacity of a single genotype to exhibit a range of phenotypes in different environments (Whitman and Agrawal, 2009), might compensate for a lack of adaptive ability and precede, or even favor, adaptive changes (Charmantier et al., 2008; Lardies and Bozinovic, 2008). Physiological plasticity can confer resilience to climate change (Chevin et al., 2010, Seebacher et al., 2015); for example, thermal plasticity appears to be inversely related to vulnerability to climate change in a range of taxonomic groups (e.g., Stillman, 2003; Calosi et al., 2008, 2010; Donelson et al., 2011; Arribas et al., 2012a). Ultimately, physiological plasticity should promote the establishment of a population in a new environment and its persistence when environmental conditions change (Ghalambor et al., 2007).

Phenotypic plasticity can be viewed as an adaptive response to environmental heterogeneity and predictability (Lardies & Bozinovic, 2008). Some studies have suggested that physiological plasticity seems to be higher in organisms living in moderately variable environments, such as temperate areas, and limited in those living in very stable (Hoffmann and Harshman, 2000) or highly variable environments (Sanders et al., 1991; Hofmann and Somero, 1995; Gaston et al., 2009; Arribas et al., 2012b; Botella-Cruz et al., 2016), meaning that species from such stable or highly variable environments may be more vulnerable to climate change than species from moderately variable ones (Stillman, 2003; Tomanek, 2009; Magozzi and Calosi, 2015).

Other studies suggest the existence of trade-offs between absolute limits and plasticity; organisms with the highest overall thermal tolerance exhibiting the lowest plasticity of this tolerance (Calosi et al., 2008; Bozinovic et al., 2011; Gunderson and Stillman, 2015).

Although these hypotheses were originally framed in the context of between-species comparisons, one could expect similar patterns in phenotypic plasticity to be found at the intraspecific level (see Gaston et al., 2009). Thus, latitudinal variation in life-history and metabolic traits amongst populations, often linked to climate and temperature variability, are ubiquitous in ectotherms (Lardies and Bozonovic, 2008). A negative relationship between mean standard metabolic rate and ambient environmental temperature is a common physiographic pattern found along latitudinal clines between populations of terrestrial insects (Addo-Bediako et al., 2002; Gaston et al., 2009). In terms of reproduction, an increased number of eggs and higher reproductive output at elevated latitudes have also been observed in some invertebrate species (e.g., Van't Land et al., 1999; Lardies and Bozonovic, 2008). Moreover egg size is, in general, inversely linked to egg number (Fox and Czesak, 2000), although in some cases (e.g., *Drosophila melanogaster*) a positive relationship between egg size and latitude has been described (Azevedo et al., 1996).

To better predict how species may respond to ongoing climate change, it is also necessary to consider the combined effect of multiple stressors from a physiological perspective (Gunderson et al., 2016; Hewitt, Ellis & Thrush 2016). In inland aquatic ecosystems, water salinity has been predicted to fluctuate widely in association with changes in temperature and precipitation patterns (Poff et al., 2002). Temperature and salinity can have a synergetic or antagonist effect on species performance (Todgham & Stillman, 2013). Thus, population persistence in dynamic and multivariate environments greatly depends on their ability to deal with the interactive effects of different stressors occurring simultaneously or sequentially over short time-scales (Gunderson et al., 2016).

In the present study we explore differences in metabolic and oviposition rates and egg size, and their plasticity in response to changes in water temperature and salinity, between core and marginal populations of the eurythermic aquatic bug *Sigara selecta* (Fieber, 1848), and go on to consider how these differences may shape population responses to ongoing global change. Based on the general patterns described for invertebrates, we predict that: 1) metabolic and oviposition rates will be higher in the northern marginal population than in the southern core one, but that eggs size should be higher in the southern core population, and 2) the northern marginal population, inhabiting moderately variable habitats, would show higher plasticity in metabolic and reproductive traits than the core population, from highly variable habitats.

2. MATERIALS AND METHODS

2.1. Study species, specimen collection, and laboratory maintenance

Sigara selecta is a eurythermic aquatic corixid that inhabits saline and brackish lentic coastal water bodies (Carbonell et al., 2012). Like most corixids, it is a benthic diving insect with a compressible gas gill, with a high storage capacity, which is renewed at regular intervals at the water surface (Popham, 1959, 1960). It is adapted to live in water bodies dominated by autotrophy (gross primary production > ecosystem respiration, see

Velasco et al., 2003; Gutiérrez-Cánovas et al., 2009), which show large daily and seasonal variation in dissolved oxygen, from being hyperoxic during the day to anoxic at night. Consequently, in nature, its gas gill is likely to be efficient at relatively low dissolved oxygen concentrations. This species, distributed in Western Europe and North Africa (Fig. 1 - Jansson, 1986; Aukema and Rieger, 1995), offers an opportunity to examine geographic variation in phenotypic plasticity of life-history and metabolic traits between core and marginal populations.

Since the experimental procedure required working with live individuals from different populations, over a short period of time to reduce seasonal effects on plasticity, two representative populations from different latitudes but similar longitude were selected. The northern population (SE England) occupies cold and more thermally stable habitats close to the northern range margin – hereafter ‘marginal population’. The southern population (SE Spain) inhabits warmer and more thermally variable sites near the center of the species distributional range – hereafter ‘core population’. (Fig. 1, Table 1). The sampling locality of the core population has a semiarid Mediterranean climate, with a mean annual air temperature of 18.1 ºC and high annual variation in temperature (28.3 ºC), with a maximum annual temperature of 33.1 ºC (mean data for the last 30 years). The northern locality of the marginal population is much cooler (mean annual air temperature 10.3 ºC), has lower annual variation in temperature (20.7 ºC) and a maximum annual temperature of 21.8 ºC (Table 1). An increase in mean temperature and water salinity, and its variation, is expected at both localities with ongoing climate change (IPCC, 2013).

We collected specimens of both populations between the end of May and early June 2014, these specimens being adults from the first spring generation (Savage, 1989; Barahona et al., 2005). Bugs were collected with a D-framed pond net with 1 mm mesh. They were later transported to the laboratory inside plastic containers filled with damp aquatic vegetation to prevent desiccation and mechanical damage during transport. Containers were kept within thermally insulated polystyrene boxes with water ice in the bottom in order to minimize thermal fluctuations as much as possible. Insects were transferred to an aquarium facility 24 h after collection. Upon arrival in the laboratory, individuals were maintained at 15 ºC with a natural photoperiod (approx. 15 h light: 9 h dark) for 24 h in a 5 L aquarium with their original water and aquatic vegetation. They were fed *ad libitum* with frozen chironomid larvae.

2.2. Experimental design

To investigate the combined effects of water temperature and salinity changes on metabolic and reproductive traits and their plasticity, an orthogonal experimental design was used, incorporating two levels of temperature (15 and 25 ºC) and salinity (10 and 35 g L^{-1}). Since previous laboratory experiences combining higher temperatures and salinities resulted in the rapid dead of individuals, we chose temperature and salinity ranges representative of the habitats where the species usually lives. The two temperatures tested were chosen as they correspond approximately to the summer average temperature for the two sampling localities respectively (http://climate-data.org_last accessed 1 November 2014 - see Table 1). The two salinities correspond to the average point of isotonicity for aquatic insects (10 g L^{-1}; Chown and Nicolson, 2004; Bradley, 2009) and one at which the species normally occurs in the field (35 g L^{-1} - Barahona et al., 2005; Carbonell et al., 2012), but at which hypo-osmotic regulation is necessary. Water of different salinities was prepared by dissolving an appropriate amount of artificial sea salt (Instant Ocean, Aquarium Systems, Sarrebourg, France) in distilled water.

2.3. Reproductive traits

Male-female pairs from each population were transferred to 100 mL containers at each salinity x temperature treatment (n = 15 pairs by treatment and population). Containers were held inside a controlled-temperature room kept at each temperature (15 or 25 ºC) with L:D 12:12 h for 7 d and specimens were fed daily with frozen chironomid larvae. If the male died before the end of the trial, it was replaced. A piece of plastic mesh was placed in each container as an oviposition substrate. Eggs laid during the first two days were not included in our estimations, to minimise the effect of females carrying eggs when collected in the field; after this time egg production was monitored daily. After eggs were counted, they were removed from the substrate and measured. Length and width of collected eggs were immediately scored using a Leica MZ8 stereomicroscope with an eyepiece micrometer (to 1 µm). Egg volume was calculated using the following formula for an ellipsoid:

$$\text{Egg volume} = 4/3 * \pi * a * b * c$$

where a = length / 2, b and c = width / 2

Fecundity plasticity was estimated as the change in magnitude of oviposition rate and egg volume between acclimation temperatures and salinities.

2.4. Metabolic rate determination

Routine metabolic rate ($\dot{M}O_2$) was determined using closed respirometry based on measures of oxygen exchange between the bug's air bubble and the surrounding water (Di Giovanni et al., 1999; Kehl & Dettner, 2009). $\dot{M}O_2$ was measured after seven days acclimation at each temperature x salinity treatment for the same individual females after the previous oviposition period (n = 12). Each chamber (10 mL blackened glass chambers, each containing one individual) was supplied with oxygen saturated water at the appropriate temperature and salinity, pre-filtered (0.22 µm vacuum filter) to remove algae and bacteria to minimize background oxygen production and respiration respectively. To standardize the volume of air in the bubble carried by the insects, individuals were allowed to replenish their air bubble at the water surface before being introduced into the chamber. In addition, to control for background fluctuations in oxygen measurements, three respirometry chambers were left empty in each trial, equipped with a magnetic flea and placed on a multi-channel magnetic stirrer (MIX 15 eco; 2mag AG, Munich, Germany) to ensure moderate mixing of water. All chambers were sealed while submerged to ensure no air bubbles were present.

Oxygen levels in the chambers were measured every 2 min using a calibrated optical O_2 analyzer (5250i, OxySense, Dallas, TX) in combination with an external probe (101, OxySense) and a fluorescent disc placed inside each chamber (Oxydot, OxySense). Although preliminary tests showed that individuals remained alive for more than two hours in respirometry chambers without a surface air space, $\dot{M}O_2$ was measured over the first 60 minutes to avoid critical hypoxic conditions. During this first hour, variation in PO_2 was linear (see Appendix S1), indicating that the critical point, at which PO_2 drastically declines, was not reached. During experiments bugs divided their time between resting and swimming. When resting, the oar-like hind legs were moved synchronously to ventilate their gas gill, this being part of the normal respiratory behavior of corixids (Popham, 1960; Matthews & Seymour, 2010). Measurements were undertaken inside temperature controlled rooms at the appropriate treatment temperature to improve thermal stability. $\dot{M}O_2$ was expressed as nmol O_2 h^{-1} STP (standard temperature and pressure) per unit wet mass (mg). To calculate $\dot{M}O_2$ the volume of individuals were estimated and subtracted from the volume of the chambers to determine the volume of water present during measurements. Upon completion of $\dot{M}O_2$ measurements, insects were removed from the chamber, blotted dry, and weighed with an electronic high-precision balance to ± 0.0001 g (MS 1225 P, Sartorius AG, Goettingen, Germany) to obtain individual wet mass.

Metabolic plasticity was determined for each population at the two studied salinities as the response of metabolic rate to changing temperature, and was expressed as Q_{10} values according to the formula:

$Q_{10} = K_1 / K_2^{10 / t1-t2}$

Where K_1 = the mean metabolic rate at temperature t_1 (15ºC)

K_2 = the mean metabolic rate at temperature t_2 (25ºC)

2.5. Data analysis

To explore how acclimation at different temperatures and salinities affected oxygen consumption, oviposition rate and egg volume in *S. selecta*, we employed Generalized Linear Models (GLM) with ‘population’ and ‘temperature x salinity treatment’ as fixed independent factors and body mass as a covariate. We assumed a Gaussian distribution and identity link function (equivalent to a 3-factor ANCOVA*).* Sidak's post-hoc tests were implemented to identify significant differences in the response variables between populations and/or treatments. In addition, to test the independent effect of temperature and salinity on response variables in each population we ran GLM tests with these environmental variables as fixed factors and weight as covariate, separately for reach population. Possible trade-offs between metabolic and oviposition rates were analyzed using Pearson's correlation tests for each temperature level and population. All statistical analyses were conducted using SPSS for Windows, version 15.0.1.

3. RESULTS

3.1. Oviposition rate

Mean oviposition rates were higher overall in the marginal population (core: 0.104 eggs day^{-1} ± 0.021; marginal: 0.408 eggs day^{-1} ± 0.079, $F = 6.51$, $P = 0.012$), which also had heavier females (core: 0.0063 g ± 0.0001; marginal: 0.0067 g ± 0.0001; $F = 4.78$, $P = 0.032$). The oviposition rate of the marginal population was significantly higher than that of the core, in the 25 ºC x 10 g L^{-1} treatment (Fig. 2, Table 2). Temperature had a significant positive impact on the oviposition rate of both populations, with higher oviposition rates at 25 than at 15 ºC. In contrast, salinity did not significantly affect oviposition rate in either of the two populations (Tables S2.1 and S2.2 in Appendix S2). Body mass had a significant positive effect on oviposition rate in the marginal population, but had no effect in the core (Tables S2.1 and S2.2 in Appendix S2). The marginal population showed higher plasticity of oviposition rate between temperatures than the core, at both salinities (Fig. 2, Table 3).

3.2. Egg volume

Significant differences in mean egg volume were found between populations in all temperature x salinity treatments, except 25 ºC x 10 g L^{-1} (Fig. 3, Table 2). Core population eggs were on average 27 % larger than those from the marginal population (Core: 0.092 mm^3 ± 0.002; Marginal: 0.072 mm^3 ± 0.002) (Table 3). Salinity and temperature significantly affected egg volume in both populations, but in different ways (significant population x treatment interaction $p<0.001$, Table 2; and temperature x salinity $p< 0.007$, Tables S2.3 and S2.4 in Appendix S2). In the core population, eggs were larger at low temperatures and high salinities (Fig. 3), whilst in the marginal population the opposite pattern was observed.

3.3. Oxygen consumption rate and metabolic plasticity

Mean oxygen consumption ranged between 21.4 ± 3.5 nmol O_2 h^{-1} mg^{-1} at 15 ºC and 10 g L^{-1} in the core population and 57.3 ± 7.5 nmol O_2 h^{-1} mg^{-1} at 25 ºC and 35 g L^{-1} in the marginal population (Fig. 4). No significant differences in oxygen consumption rates were found between core and marginal populations, although there were significant differences across treatments (Table 2). In both populations, oxygen consumption rate was significantly increased in the highest salinity and temperature treatment (Fig. 4). Maximum mean $\Delta\dot{M}O_2$ was 105.94 nmol O_2 h^{-1} mg^{-1} in the marginal population and 125.53 nmol O_2 h^{-1} mg^{-1} in the core population between the 15 ºC x 10 g L^{-1} and 25 ºC x 35 g L^{-1} treatments. In the marginal population, increases in both salinity and temperature produced significant increases in metabolic rate, whilst in the core population only higher salinity increased metabolic rate significantly (Tables S2.5 and S2.6 in Appendix S2).

Q_{10} values were higher at salinity 35 than at salinity 10 in both populations (Table 3) and the marginal population showed higher Q_{10} values than those of the core at salinity 35. No significant relationships were found between metabolic and reproductive traits (Appendix S3).

4. DISCUSSION

The populations that inhabit the margins of a species' distributional range are likely to be critically important in determining its responses to ongoing climate change (Thomas et al., 2001; Iverson et al., 2004; Travis and Dytham, 2004). Our results reveal that the northern marginal population of *S. selecta* was more sensitive to changes in temperature and salinity than the core population studied, showing higher phenotypic plasticity in its metabolic and reproductive traits. This evidence could have positive implications when coping with future environmental change.

The life history traits of aquatic insects are mainly dependent on environmental temperatures (Sweeney, 1984), particularly fecundity, growth rate and adult body size (Vannote and Sweeney, 1980). Fecundity typically increases with temperature up to a threshold causing a shift in energy allocation away from reproduction into maintenance and repair (Huang et al., 2007; Hercus et al., 2003; Massamba-N´Siala et al., 2012). This positive effect on oviposition rate was seen in both populations at 25 ºC, particularly in the marginal population which showed greater plasticity in this trait. Such a difference in fecundity plasticity between populations could reflect local adaptation to environmental temperature variation, with both wider daily and seasonal thermal variation, and a longer reproductive period in the core population than in the northern marginal one. The northern marginal population may therefore be adapted to maximize egg production in the shorter reproductive period (from May to the end of summer) when temperatures are optimal (mean monthly temperature >10 ºC, Barahona et al., 2005). On the other hand, the core population can extend its breeding period, with a lower oviposition rate, but a higher annual reproductive output overall. Such a finding is in accordance with known latitudinal and climatic variation in voltinism in aquatic insects (Sweeney, 1984). English corixids are typically univoltine or in some cases bivoltine (Savage, 1989), whilst south-eastern Iberian populations of *S. selecta* breed between March and October, with four generations a year (Barahona et al., 2005). Differences in fecundity between the two populations may be partially explained by weight differences between females. Those from the marginal population are approximately 9 % heavier than those from the core population, probably due to a longer period of nymphal development. Such larger size in colder conditions is normally attained by a prolonged growth period overcompensating for slow growth at low temperatures (Kozłowski et al., 2004; Gaston et al., 2009).

As expected we found an inverse relationship between the number and size of eggs, with the eggs of the core population being approx. 27 % larger in volume and more plastic in size in response to increases in salinity than those of the marginal population. The semiarid Mediterranean climate experienced by the core population in southeast Spain means that occupied water bodies are subject to frequent droughts and sudden increases in salinity and/or temperature (Millán et al., 2006). In such areas larger eggs may be more resistant to drought than smaller ones, since the former have more stored water and a lower surface area/volume ratio (Le Lagadec et al., 1998; Lapinski and Tschapka, 2014).

Contrary to expectations, no inter-population differences in metabolic rate were found within the temperature and salinity ranges examined. The metabolic rates of both populations increased at high temperatures and salinities. At salinities above the iso-osmotic point (10 g L^{-1}), there is an increase in the cost of osmoregulation in most aquatic insects (Nelson et al., 1977). However, this potential osmoregulation cost did not result in a trade-off with oviposition rate under our experimental conditions. Despite this, at higher temperatures and salinities (above 25 ºC and 35 g L^{-1}), physiological homeostasis could have negative consequences on fitness, decreasing the amount of resources/energy allocated to reproduction and maintenance (Folguera et al., 2011). Where such a threshold sits for this species could not be determined in our experiments, however, acclimation to salinities above those employed here (e.g. 50-75 g L^{-1}) rapidly led to the death of animals in the laboratory.

In hyperosmotic media (e.g., 35 g L^{-1}), the metabolic rate of the marginal population was more sensitive to changes in temperature than that of the core, as the former showed higher Q_{10} values, indicating greater metabolic plasticity (Calosi et al., 2005, 2007). Population differences in metabolic plasticity could reflect adaptations to environmental variability and predictability, as have previously been observed in interspecific comparisons (Stillman, 2003; Tomanek, 2009). The marginal population normally experiences narrower variation in temperature (see Table 1) and apparently compensates for temperature change by increasing metabolism to a greater extent. In contrast the core population, which experiences a wider and more unpredictable range of temperatures, appears to be less sensitive to changes in temperature. Our results are in accordance with the general pattern observed in terrestrial animals, with the metabolic rates of species from warm environments being less sensitive to temperature variation than those from cooler areas (Seebacher et al., 2015). It seems that this air-breathing aquatic bug behaves more like a terrestrial than an aquatic organism in this regard, in line with its terrestrial evolutionary origin (Pritchard et al., 1993; Bozinovic et al., 2011).

Although there is no clear intra-specific pattern between metabolic acclimation ability and climate or latitude, the low metabolic and reproductive plasticity found in the core population is similar to the low plasticity observed in the upper thermal limit of other saline insects such as aquatic Coleoptera (Arribas et al., 2012b; Botella-Cruz et al., 2016). The higher plasticity in metabolic and reproductive traits seen in the marginal population of *S. selecta* may provide resilience against the effects of ongoing climate change. Core populations, which currently experience more extreme and variable temperatures, but show lower plasticity, might be more resistant to environmental changes within their tolerance ranges. Outside such tolerance ranges, however, these populations will be more vulnerable to global warming.

The implications of the patterns we uncover for population persistence or range expansion in this species could be profound. An increase of mean temperature at the northern edge of the distributional range of *S. selecta* is likely to increase the degree of climatic suitability or habitat quality in both currently occupied and new locations. Our results suggest that warming in these northern areas could increase oviposition rate and extend the oviposition period, increasing reproductive output, and allowing both persistence and range expansion. Such increases in temperature could incur a metabolic cost, but this does not lead to an apparent trade-off with fecundity, at least below 25 ºC. In addition, higher temperatures may lead to an increase in dispersal flights in this insect

(Kirkpatrick et al., 1997; Thomas et al., 2001), further facilitating range expansion. Whether the plastic responses seen in our study could be sustained in the long term, considering potential costs associated with them remains unclear, however. In marked contrast to those close to the northern range edge, core populations, despite being more resistant to environmental fluctuations, might be more sensitive to rapid and intense warming events, perhaps driving a northward retreat of the species southern range limits (Hughes, 2000; Parmesan, 2006).

5. CONCLUSIONS

The population-specific responses of *S. selecta* to environmental change are complex and may be mediated by differences in phenotypic plasticity related to the environmental variability experienced in nature. When comparing the studied populations of *S. selecta* at the center and edge of its geographical range, the northern marginal population showed higher oviposition rates, smaller eggs and higher levels of metabolic and fecundity plasticity than the core population. The higher plasticity of the marginal population may increase its fitness in current habitats and facilitate its expansion northwards with climate warming. Whilst the core population seems less sensitive to changes in temperature and salinity it could be prone to extinction in current localities if temperature and salinity changes exceed physiological tolerance limits, leading to a northward retreat of the species southern range limits. Further extension of this experimental study, considering several populations of *S. selecta* in the marginal and core areas within the species geographical range are of special concern to corroborate the generality of the pattern found.

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SUPPORTING INFORMATION

Metabolic and reproductive plasticity of core and marginal populations of the eurythermic and saline water bug *Sigara selecta* (Hemiptera: Corixidae) in a climate change context.

J.A. Carbonell, D.T. Bilton, P. Calosi, A. Millán, A. Stewart & J. Velasco

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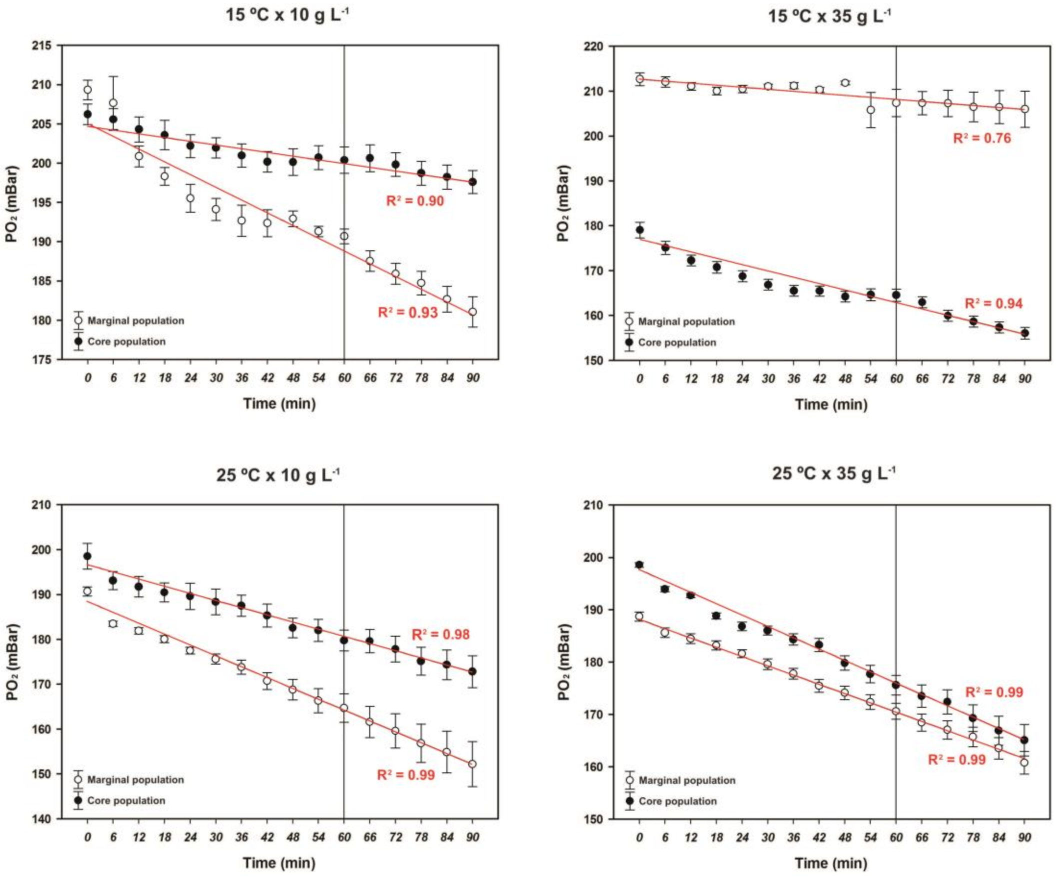
Appendix S1. Changes in PO_2 along experiments for the marginal and core populations at the four temperature x salinity treatments.



Appendix S2. GLM results at population level. GLM assuming Gaussian distribution and identity link function.

S2.1. Effects of temperature, salinity and their interaction on oviposition rate for the core population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	0.153	4	2.012	0.110
Intercept	0.022	1	1.171	0.285
Weight (cov)	0.063	1	3.323	0.075
Temperature	0.096	1	5.082	**0.029**
Salinity	0.170	1	0.876	0.354
Temperature x salinity	0.005	1	0.245	0.623
Error	0.816	43		

S2.2. Effects of temperature, salinity and their interaction on oviposition rate for the marginal population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	5.962	4	7.854	< 0.001
Intercept	2.409	1	12.696	0.001
Weight (cov)	3.399	1	17.914	**< 0.001**
Temperature	2.644	1	13.934	**0.001**
Salinity	0.002	1	0.008	0.929
Temperature x salinity	0.314	1	1.656	0.205
Error	8.160	43		

S2.3. Effects of temperature, salinity and their interaction on eggs volume for the core population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	0.003	3	7.112	0.001
Intercept	0.342	1	2384.413	< 0.001
Temperature	0.001	1	5.582	**0.024**
Salinity	0.001	1	7.411	**0.010**
Temperature x salinity	0.001	1	8.341	**0.007**
Error	0.005	36		

S2.4. Effects of temperature, salinity and their interaction on eggs volume for the marginal population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	0.004	3	27.308	< 0.001
Intercept	0.213	1	4536.798	< 0.001
Temperature	0.000	1	8.261	**0.007**
Salinity	0.003	1	65.324	**< 0.001**
Temperature x salinity	0.000	1	8.338	**0.007**
Error	0.002	36		

S2.5. Effects of temperature, salinity and their interaction on oxygen consumption rate for the core population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	6585.627	4	2.946	0.030
Intercept	3745.880	1	6.703	0.014
Weight (cov)	1057.905	1	1.893	0.178
Temperature	219.456	1	0.393	0.535
Salinity	3065.139	1	5.485	**0.015**
Temperature x salinity	232.374	1	0,416	0.523
Error	18999.763	34		

S2.6. Effects of temperature, salinity and their interaction on oxygen consumption rate for the marginal population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	1019.602	4	5.182	0.003
Intercept	1899.266	1	9.653	0.004
Weight (cov)	558.252	1	2.837	0.104
Temperature	1027.307	1	5.222	**0.030**
Salinity	1667.542	1	8.476	**0.007**
Temperature x salinity	250.740	1	1274.000	0.269
Error	196.744	27		

Appendix S3. Pearson product-moment correlation coefficients between metabolic rates and oviposition rates for core and marginal populations of *S. selecta* at each studied temperature.

	15 ºC		**25 ºC**	
	Cor. Pearson	**Sig.**	**Cor. Pearson**	**Sig.**
Core population	-0.105	0.866	0.320	0.439
Marginal population	-0.698	0.190	-0.184	0.636