Variation in thermal tolerance response associated with geographic location during early development of the neogastropod *Ocenebra erinaceus* (Linnaeus, 1758)

Maria Loreto Mardones[1]*, Phillip B. Fenberg[1], Sven Thatje[1], Chris Hauton[1]

1. School of Ocean and Earth Science, National Oceanography Centre, University of Southampton, Southampton SO14 3ZH, UK

Corresponding author: mlmv1c20@soton.ac.uk

Address: School of Ocean and Earth Science, University of Southampton, National Oceanography Centre Southampton, European Way, Southampton SO14 3ZH, UK

Keywords: Neogastropoda, *Ocenebra erinaceus*, intracapsular development, thermal tolerance, intraspecific differences.

Abstract

Environmental temperature plays an important role in shaping the distribution and abundance of marine ectothermic organisms. As a general rule, larvae and juveniles are more sensitive to thermal stress than adults and, as a consequence, represent key life stages that determine in part the geographic range of a species. Identifying critical thermal limits during ontogeny allows for the prediction of the potential impacts of climate warming on the distribution of marine ectotherms. However, thermal tolerance - and therefore the potential to meet the challenge of warming- is known to vary at population scale for many species. In order to fully appreciate a species' future under climate warming, multiple populations studies from different thermal environments are necessary. In this study, we compared the thermal tolerance response during the intracapsular development of the marine gastropod *Ocenebra erinaceus* between two geographically separated populations: one from the middle (Solent, UK) and another from the south of the species' geographic range (Arcachon, France). The results show that the thermal tolerance response was influenced by geographic origin. Embryos from the relatively warm-water southern population (France) show a warm-eurythermal tolerance window with optimal temperatures between 12 and 18 °C. On the contrary, embryos from the cold-water northern population (UK) exhibit a narrow, warm stenothermal, thermal tolerance window with optimal temperatures between 14 and 16 °C. In both populations, temperatures outside of the thermal range cause lethal and sub-lethal effects. Importantly, previously observed dispersal polymorphism was not observed at hatching time in either population in our study. Our study demonstrates that during early developmental stages, embryos are adapted to local thermal conditions and that they live very close to their upper thermal limits. Temperatures outside this range cause detrimental and

contrasting effects on embryonic development of *O. erinaceus*, implying that the effects of future warming will depend on the population response to local environmental history. Our results suggest that global warming could shift the geographical distribution range of *O. erinaceus* poleward.

1. Introduction

Temperature is the main environmental driver controlling the physiology, ecology and distribution patterns of terrestrial and marine species (Castañeda et al., 2004; Jones et al., 2009; Somero, 2005). As marine invertebrates are unable to regulate body temperatures independently from the marine environment (i.e. ectothermic), nearly all physiological and behavioural traits are sensitive to temperature (e.g. metabolism, locomotion, immune function, foraging ability, rates of feeding and growth; Angilletta et al., 2002). Within an organism's thermal tolerance range, the performance rate increases as temperature increases from a critical minimum to an optimum (Schulte et al., 2011). However, temperatures outside of the optimal thermal range cause a decrease in the physiological performance (e.g. aerobic metabolism) due to oxygen limitation (i.e. the mismatch between oxygen supply and demand), which leads to a shift from aerobic metabolism to an anaerobic mode, until the ultimate failure of the whole-organism (Pörtner, 2002, 2010). Physiological thermal capabilities of organisms define the thermal tolerance limits and are predictive of their distribution patterns (Somero, 2002). Hence, the identification of these thermal limits can provide important insight into the limits of distribution ranges of marine species and, at the same time, describe the potential impact of future warming on shifts in distribution ranges.

The thermal tolerance range in ectothermic organisms can vary across species geographic distribution and among populations of a single species (Sunday et al., 2011). Latitudinal

thermal gradients can influence the thermal tolerance response, especially in terrestrial ectotherms where the thermal range increases as latitude (from tropical to polar) or environmental variation increases (Gaitan-Espitia et al., 2016; Yu et al., 2018). However, marine ectotherms show a lower rate of increase in thermal tolerance with latitude and a poleward decrease at both upper and lower thermal limits (Sunday et al., 2011). Persistent environmental variations (e.g. tidal regimes, wave action, local temperature) can promote physiological genetic adaptations within a species distribution range, resulting in a mosaic of populations variously physiologically adapted (Kelly et al., 2012, Kuo and Sanford, 2009; Sanford and Kelly, 2011). Thus, it is important to understand whether the thermal tolerances can vary among populations across a species geographic range to predict whether the potential impact of warming acts differently on each population.

Global sea temperatures are rising, producing a significant shift in species distributions and promoting extinction of more thermally-sensitive species (Burrows et al., 2011; Harley et al., 2006; Helmuth et al., 2002; Sunday et al., 2015). Early ontogenetic stages are considered as the bottleneck in the life of cycle of marine organisms as they are generally more thermally sensitive than adults from the same population (Anger et al., 2003; Sanford et al., 2006). This is particularly evident for gastropods species that deposit their embryos in external structures (i.e. gelatinous mass or capsules) on the rocky shore, until they hatch as swimming larvae or juveniles (Pechenik, 1979). Encapsulation can confer protection during the intracapsular development against osmotic changes, UV exposure and predation (Przeslawski, 2004). However, previous reports have shown that high temperatures increase hypoxic conditions inside capsules producing high mortality and abnormalities (Fernández et al., 2006; Smith et al., 2013). Thus, if the external environmental conditions (e.g. heat waves) exceed the

88 physiological limits, encapsulated embryos cannot move and find alternate suitable conditions;
89 thus, encapsulated species can be at potential risk under warming. However, little is known
90 about the thermal tolerances in encapsulated embryos and whether the thermal tolerances
91 can vary among populations across a species' geographic range.

92 To study the thermal tolerance of an encapsulated species, the temperate neogastropod
93 mollusc *Ocenebra erinaceus* was chosen as a model. This species is a direct developer; females
94 enclose their embryos in capsules and leave them for up to three months on rocky shores
95 (Smith et al., 2015) or on rocks in the shallow subtidal. During intracapsular development, the
96 species develops through eight ontogenetic stages from egg to different veliger stages. At
97 hatching, swimming larvae emerge from the capsules, spending a few days in the plankton
98 before they settle as juvenile (Gibbs, 1996). However, Smith et al. (2015) reported dispersal
99 polymorphisms, meaning that from the same egg mass two different larval forms might
100 emerge. The authors reported that a proportion hatched as swimming larvae whilst others as
101 crawling juveniles; at 15 °C (experimental temperature), 14% of hatchlings were swimming
102 larvae (Smith et al., 2015). Dispersal polymorphism seems to be a very advantageous
103 reproductive strategy in terms of increasing the dispersal connectivity between populations
104 and also by increasing the possibility to find more suitable habitats.

105 In this study, we identified the thermal tolerance response of early stages of *O. erinaceus* and
106 determined if the thermal tolerance is influenced by their geographical origin. To determine
107 intraspecific differences, two shallow subtidal populations from different thermal environment
108 were compared: one from the middle and another from the south of the species' geographic
109 range. We predicted that embryos from relatively warm waters would exhibit wider thermal
110 tolerance windows and high survival as a physiological adaptation to the warmer seawater

111 temperatures that they experienced through the year. The findings of this study will contribute
112 to our understanding of physiological adaptations to local thermal conditions and provide
113 insights as to how early ontogenetic stages from geographically distinct populations will be
114 impacted by ocean warming.

115 ## 2. Material and methods

116 ### 2.1. Study sites and female maintenance

117 To study the thermal tolerance response, females of *Ocenebra erinaceus* were collected form
118 the shallow subtidal via trawling from the Solent, UK (50° 51' N, 001° 21' W; hereafter named:
119 northern population) between February-March 2016 (9 – 10 °C) and from a commercial oyster
120 bed in Arcachon, France (44° 41' N, 001° 11' W; hereafter named: southern population) in
121 October 2017 (14 – 16 °C). After sampling, females were transferred to the National
122 Oceanography Centre, Southampton (NOCS), UK to aquarium conditions. Laboratory thermal
123 conditions were designed according to the sea-water temperatures that females experienced
124 in the field to avoid an effect of acclimatization to laboratory conditions (see Mardones et al.,
125 2020a for further details). Average monthly temperatures were calculated for each population
126 from annual databases available during three consecutive years 2014 – 2016 (for the Solent:
127 XAUK buoy, https://stormcentral.waterlog.com/public/XAUKBuoy; and for Arcachon: REPHY
128 dataset 1987-2016 (http://doi.org/10.17882/47248). As specimens in the northern population
129 were collected near to aquarium facilities, northern females were maintained in laboratory
130 conditions for three months before the beginning of capsule laying process at: March 8 ± 1 °C,
131 April: 11 ± 1 °C and May: 14 ± 0.3 °C. On the contrary, as southern females were collected
132 during winter months, females were maintained in the laboratory until the laying capsule
133 process began at the same temperatures that they experience in Arcachon (October: 17 ± 1 °C;

November: 15 ± 1 °C, December: 12 ± 1 °C; January: 10 ± 1 °C, February: 10 ± 1 °C; March: 11 ± 1 °C). Salinity was relatively constant in both populations with values ranging between 32.5 and 34.0. Females from both populations were fed *ad libitum* with mussel and oysters 3 times per week. In the northern population, fifteen females (36.0 ± 5.8 mm of shell size) started to lay capsules between April-May 2016 and eighteen females (37.2 ± 2.9 mm of shell size) from the southern population started to lay capsules between February-March 2017. Fecundity (i.e. the total number of eggs per egg mass) was 1402 ± 657 and 2076 ± 641 eggs per egg mass in the north and southern population, respectively.

2.2. Egg masses collection and maintenance

After one or two days of egg laying, 2-3 egg masses from each population (n = 20 -25 capsules per egg mass) were transferred to one of six experimental temperature treatments (10, 12, 14, 16, 18 and 20 °C). For the northern population, high mortalities were observed at 18 °C; thus, embryos were not exposed to 20 °C. Egg masses were maintained individually in 1.8 L aquariums with filtered seawater (1 µm, 33-35 salinity) under constant aeration, and 100% water changes were conducted 3 times per week. Each week, 1-2 capsules were dissected to quantify: developmental rates, embryo size, capsular survival and proportion of abnormal embryos. Intracapsular ontogenetic stage was determined according to Smith et al. (2015). Number and embryo size (i.e. maximum shell length) were measured under microscope using photographs recorded with a digital camera attached to a microscope (x100 magnification; LEICA MZ16). A capsule was considered dead when capsules developed an external purple colour or when no larval activity was found. Dead capsules were dissected, and the ontogenetic stage was recorded. Developmental abnormalities were identified when some of the embryos inside the capsule showed shell malformations.

The metamorphosis time (i.e. the time that swimming late-pediveliger veliger larvae spent in the water column until they settled as a crawling juvenile) was evaluated in three capsules from each egg mass exposed to 14, 16 and 18 °C in the northern population and 14, 16, 18 and 20 °C in southern population (see Mardones et al., 2020a for further details). Capsules were maintained individually in small aquaria of 0.25l to observe the time to metamorphosis after hatching. Once the hatching process had begun, larvae were exposed to light for 15 minutes under a dissecting microscope (x40 magnification) to assess whether larvae had completed metamorphosis to juvenile crawling stage. The presence of velar lobes was considered as incomplete metamorphosis. The number of days to reach complete metamorphosis was recorded.

2.3. Statistical analysis

The effects of temperature on the survival and embryonic development (i.e. abnormalities) were analysed with one-way ANOVA followed by Tukey *post hoc* analysis in each population. Prior to analysis, the percent survival and abnormal capsules was arcsine transformed. The effects of temperature on the intracapsular development time was analysed by two-way ANOVA followed by Tukey *post hoc* analysis, with temperature and population as factors. Normality and homogeneity of variance were confirmed before respective ANOVA's analysis and statistical significance was identified at $p < 0.05$. However, when these assumptions were not meet, a non-parametric analysis Kruskal-Wallis analyses of variance by ranks followed by Dunn's method *post hoc* analysis was used to compare the metamorphosis time in both populations.

3. Results

3.1. Temperature effects on intracapsular development and embryo size

In the current study, *O. erinaceus* exhibited a mixed development with every embryo passing through a pelagic and benthic phase. During intracapsular development, *O. erinaceus* showed six ontogenetic stages: egg, trochophore, early veliger, veliger, pediveliger and late pediveliger stage (Fig. 1a-f; see appendix S1 for developmental rates and embryo size, Table S1 and S2). During hatching, larvae emerged as a swimming late-pediveliger (Fig. 1g) and depending on rearing temperatures, larvae spent hours or days in the plankton. Dispersal polymorphism was not observed at hatching in the Solent or Arcachon population. 100% of the embryos hatched as swimming late-pediveliger larvae. After that, swimming larvae lost their velar lobes completely and then settled as crawling juveniles (Fig. 1h).

Temperature and geographic origin produced a significant effect on the intracapsular time of *Ocenebra erinaceus* (Two-way ANOVA, Population: $F_{(1,23)}=5.9$, $p < 0.05$; Temperature: $F_{(3,23)}=470.6$, $p < 0.05$; P x T: $F_{(3,23)}=10.7$, $p < 0.05$; Fig. 2). Intracapsular time was successful between 10 and 20 °C in the southern population, and between 12 and 18 °C in the northern population. In general, as temperature increased the developmental time decreased. At low temperatures (10 – 12 °C), development was slower for the southern than in the northern population, taking approximately 140 days and 100 days, respectively. At high temperatures, development was similar and faster in embryos from both populations, taking approximately 45 and 30 days at 18 and 20 °C, respectively.

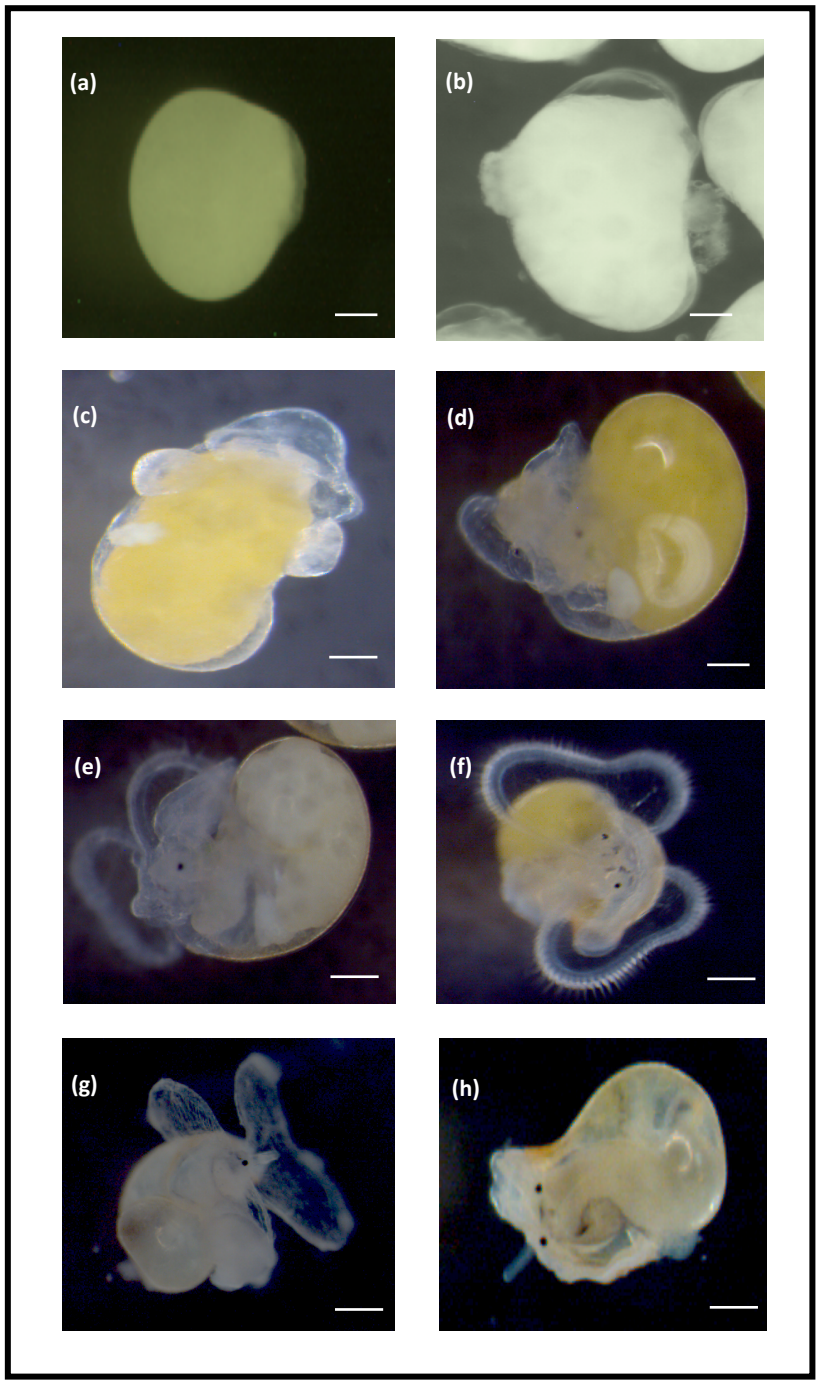
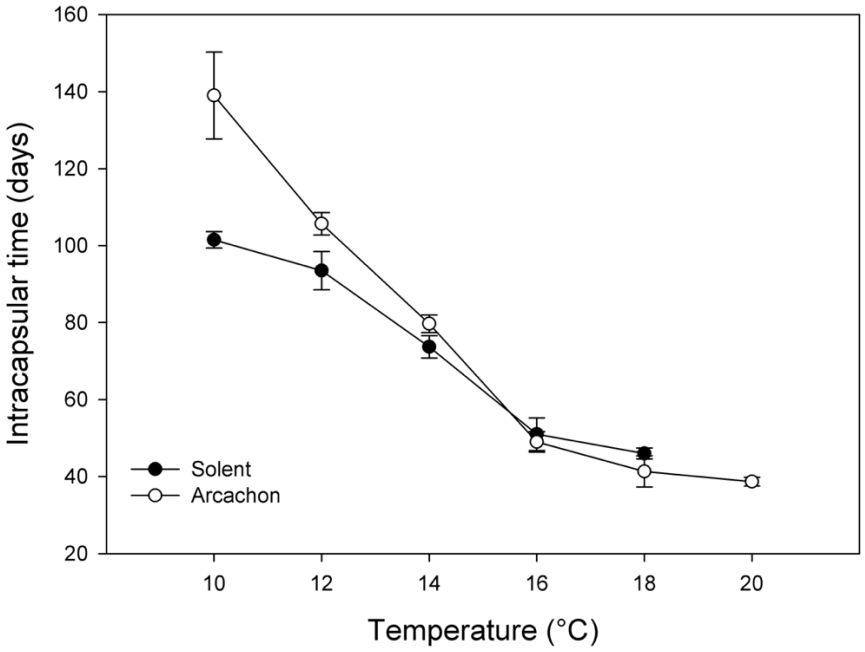


Figure 1. Ontogenetic stages during the intracapsular development of *Ocenebra erinaceus*. (**a**) Egg; (**b**) Trochophore; (**c**) Early veliger; (**d**) Veliger; (**e**) Pediveliger; (**f**) Late-pediveliger; (**g**) Swimming late-pediveliger; (**h**) Crawling juvenile. Scales bars: (a-g) = 100 µm, (h) = 250 µm



220

221 Figure 2. The effect of temperature on the intracapsular developmental time of embryos of
222 *Ocenebra erinaceus* from the Solent (UK) and Arcachon (France) populations (i.e. northern and
223 southern population, respectively). Embryos were exposed to experimental temperatures
224 between 10 and 20 °C. In the northern, development was not fully completed at 10 - 12 °C
225 (Please refer section 3.2). Values are given as mean ± standard deviation (SD). n = 3 egg masses
226 per temperature treatment.

227

228

229

230 3.2. Temperature effects on survival and abnormalities in development

231 Survival and percent normal development during intracapsular development differed between
232 temperature treatments and populations. In the northern population, embryo survival was
233 significantly affected by temperature (One-way ANOVA, $F_{(4,14)}$=24.85, $p < 0.05$; Fig. 3a). Survival
234 was significantly lower at 10 and 18 °C with values of 50 – 40%, respectively. Optimal
235 temperatures for intracapsular development were observed between 14 and 16 °C. On the
236 contrary, temperature did not significantly affect survival in the southern population; high
237 survival (100 - 70 %) was observed for all temperature treatments (One-way ANOVA,
238 $F_{(5,17)}$=1.77, $p > 0.05$; Fig. 3b).

239 The percentage of capsules with embryos with normal development was not significantly
240 different between temperature treatments in the northern population (One-way ANOVA,
241 $F_{(4,14)}$=1.03, $p > 0.05$; Fig. 3c). On the contrary, southern embryos exhibited ~20 – 25% of
242 abnormalities (i.e. shell deformations) during the development at both extremes of the
243 experimental range (One-way ANOVA, $F_{(5,17)}$=6.24, $p < 0.05$; Fig. 3d), whilst normal embryonic
244 development was observed between 12 and 18 °C.

245 Time To Metamorphosis (TTM) (i.e. the time that swimming late-pediveliger larvae spent in
246 the column of water until they metamorphosed into juvenile stage) was affected by
247 temperature and geographic origin. In the northern population, TTM was significantly affected
248 by temperature (Kruskal-Wallis by ranks, H= 11.18, $p < 0.05$; Fig. 3e). Fifty percent of swimming
249 late-pediveliger exposed to 16 or 18 °C spent more time in the pelagic environment compared
250 with larvae maintained at 14 °C. Swimming late-pediveligers settled before 24 hours at 14 °C;
251 however, at higher temperature treatments (16-18 °C) this took approximately 60 hours. On
252 the contrary, the MMT of southern swimming larvae increases with increasing temperature

253 but was not significant (Kruskal-Wallis by ranks, H= 5.66, $p > 0.05$; Fig. 3f). The time that
254 southern larvae spent in the plankton was not affected by temperature treatments with values
255 ranging between 24 and 48 hours (Dunn's *post hoc* analysis).

256

257

258

259

260

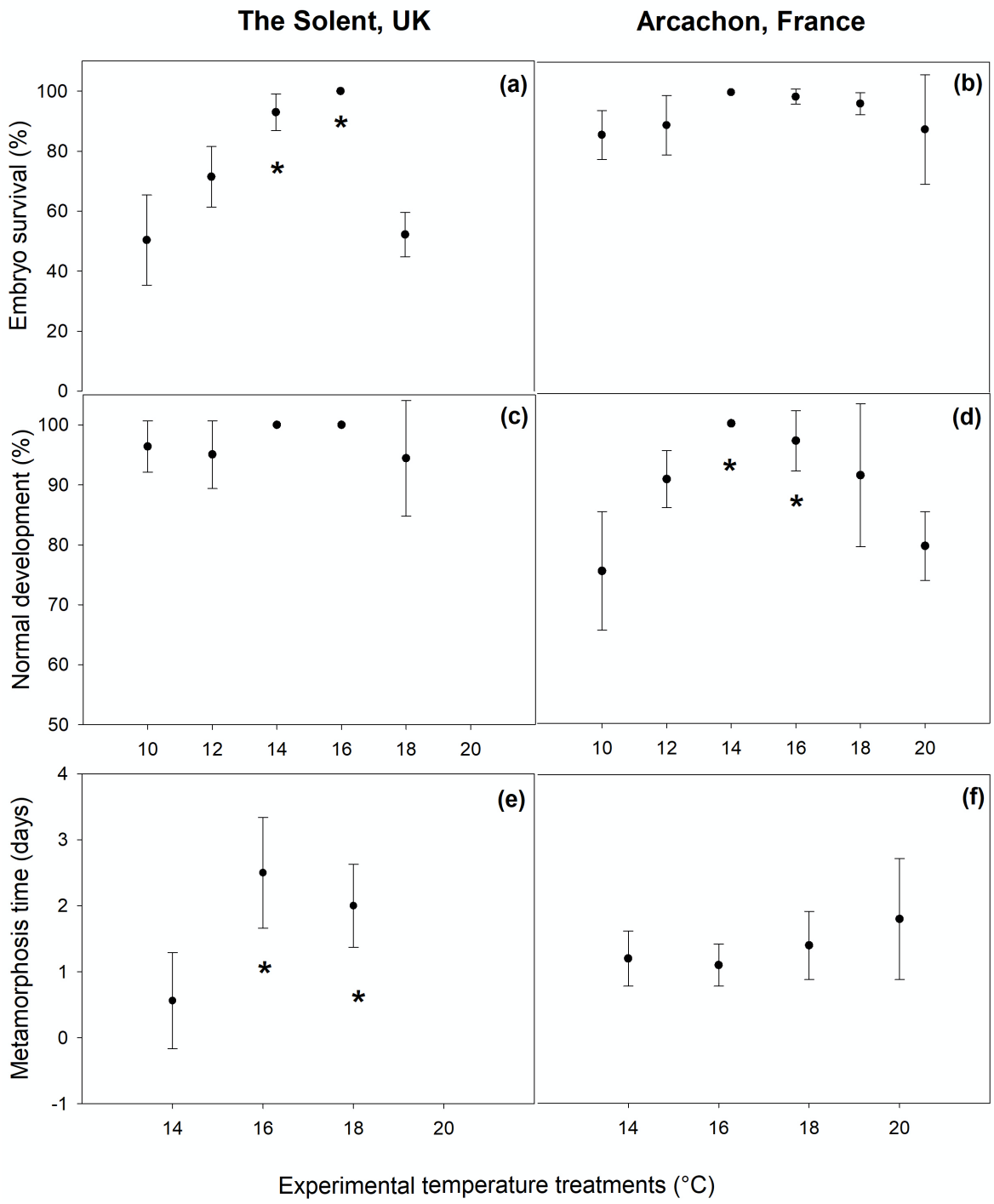


Figure 3. Effects of temperature on the percentage of embryo survival (**a-b**), embryos with normal development (**c-d**) and the metamorphosis time (e-f) in: the Solent (**a, c, e**; northern population) and Arcachon (**b, d, f**; southern population). (*) mean significant differences between treatments. Values are given as mean ± standard deviation. n = 3 egg masses per temperature treatment for embryo survival and normal development. n = 9 – 10 capsules (48 embryos approximately per capsule) per temperature treatment for metamorphosis time.

4. Discussion

Environmental temperature plays an important role in shaping the distribution and abundance of marine organisms (Jones et al., 2009; Somero, 2005). This could be more pronounced during early stages, as they exhibit greater thermal sensitivity and, as a consequence, can determine in part the geographic range of a species. The aim of this study was to identify whether geographic origin has an impact on the thermal tolerance response of encapsulated embryos of a shallow subtidal gastropod species. We found that the thermal response of early stages of *Ocenebra erinaceus* was influenced by local thermal conditions that they experienced during the intracapsular development, which led to intraspecific differences in their thermal limits and optimum temperatures. These differences could be a result of physiological and genetic adaptions to local environmental conditions (Mardones et al., 2020b); however, from our results we cannot rule out possible influences of phenotypic effects.

4.1. Temperature effects on intracapsular development and dispersal polymorphism

In both populations, the encapsulation period was highly correlated with the rearing temperatures. At 10 – 12 °C, the intracapsular development was slower than at 18 – 20°C. Exposure to low temperatures produced similar results in other marine species. Strathmann and Chaffee (1984) showed that low environmental temperatures might decrease metabolic rates in embryos because the oxygen diffusion coefficients decreased and viscosity increased in the intracapsular fluid, limiting the transport of oxygen inside of capsules. In this study, the prolonged intracapsular development exhibited by embryos of *O. erinaceus* could be a response to a reduction in their metabolic rates and oxygen deficiency. At high temperatures (18 – 20 °C), development proceeded more quickly as was observed in this study and also it

290 has been reported in other species such as *Buccinum undatum* (Smith et al., 2013) and *Thais*
291 *haemastoma* (Roller and Stickle, 1989).

292 In this study, *Ocenebra erinaceus* did not exhibit dispersal polymorphism (i.e. crawling and
293 swimming morphs at hatching) during the hatching process in both populations. Six
294 ontogenetic stages were observed during the intracapsular development (i.e. between egg and
295 late-pediveliger stage). At the end of the intracapsular development, development was
296 synchronous and larvae passed through a pelagic late pediveliger stage in all experimental
297 conditions. This result contradicts observations made by Smith et al. (2015). At the same
298 developmental time, Smith et al. (2015) observed an asynchronous development: larvae with
299 pronounced velar lobes (i.e. swimming late-pediveliger) and larvae without velar lobes
300 (considered as pre-crawling juveniles). In this study, after hours or days, depending of the
301 rearing temperature, larvae lost their velar lobes and settled as crawling juveniles. Smith et al.
302 (2015) reported that 14% of larvae hatch as swimming late-pediveliger and 86% of larvae hatch
303 as crawling juveniles (i.e. dispersal polymorphism) in the northern population. However, the
304 result of the current study agrees with Gibbs (1996) who reported that larvae of *O. erinaceus*
305 hatch with a short planktonic phase of up to five days before settling as a juvenile. In other
306 species from the same genus, direct development in *O. inornatus* (Martel et al., 2004) and
307 mixed development (i.e. offspring undergo both a non-pelagic and pelagic stages during
308 development) in *O. pulsoni* (Amio, 1963) have been reported, but there are no previous
309 reports that indicate the presence of dispersal polymorphism in this family. The differences
310 between our study and observations by Smith et al. (2015) could be methodological. In this
311 study, when swimming larvae started to hatch, they were exposed to light for approximately
312 15 minutes to observe how larvae displayed the velar lobes and started to swim. However,

313 environmental maternal conditions can influence the presence of dispersal polymorphism (e.g.
314 temperature; Clemens-Seely and Phillips 2011); thus, from this study, the results found by
315 Smith et al. (2015) cannot be completely discounted.

316 4.2. Thermal tolerance of northern and southern populations

317 Thermal tolerance response during early stages was influenced by the geographic origin and
318 showed an inverse relationship between thermal latitude range and thermal tolerance.
319 Embryos from the lower latitude population (southern population, 44° 41'N) were
320 physiological more warm-adapted than embryos from the higher latitude (northern
321 population, 50° 51' N; Fig. 3). In the southern population, the experimental range of
322 temperatures between 10 and 20 °C did not affect the survival of embryos, which reflects upon
323 the temperatures that they experienced in the field between 12 and 18 °C (Mardones et al.,
324 2020a). However, temperatures outside of the natural thermal range (10 and 20 °C) produced
325 lethal effects. Abnormalities (i.e. shell malformations) during development increased at both
326 ends of the thermal tolerance range (10 – 20 °C), which established a thermal tolerance
327 window of this population between 12 and 18 °C. Abnormalities during development have
328 been associated with elevated temperatures (e.g. shell malformations and lysis in embryos
329 from the giant clam *Tridacna gigas*, Enricuso et al., 2019) and this is particularly evident in
330 encapsulated species where temperature and oxygen are critical for development. Low oxygen
331 diffusion at extreme temperatures (i.e. low and upper thermal limits) affects the diffusion of
332 oxygen through the capsule wall causing abnormalities during the development or the
333 presence of empty shells (Fernández et al., 2006; Smith et al., 2013).

On the contrary, extreme temperatures reduced the survival by ~50% at both ends of the temperature range (10 – 18 °C) in the Solent embryos. No abnormalities were observed during the intracapsular development because embryos could not survive at extreme temperatures. In the field, northern embryos experience colder temperatures than the southern population with values ranging between 13 and 16 °C (Mardones et al., 2020a). Therefore, the high survival observed between temperatures 14 and 16 °C is coincident with the temperatures that they normally experienced. However, temperatures outside of the natural thermal range produced deleterious effects, which establish a very narrow tolerance window with thermal limits between 14 and 16 °C. This result is in contradiction to the climate variability hypothesis (CVH), which states that thermal adaptation is proportional to the magnitude of the environmental variation; thus, individuals from a species inhabiting at high latitudes require broader tolerance ranges than individuals inhabiting lower latitudes (Yu et al., 2018, Gaitan-Espitia et al., 2016). Previous reports have suggested that thermal tolerance variation among populations can be a result of genetic adaptations to local thermal conditions (Gleason and Burton, 2013; Jansen et al., 2007). For example, Reitzel et al. (2013) conducted a common garden experiment and they evidenced that adult and developmental stages of the temperate sea anemone *Nemastotella vectensis* were locally adapted. They found that low latitude populations have higher thermal tolerance as a consequence of the warm temperatures that they experienced. Zippay and Hoffmann (2010) found that veligers of the gastropod *Nucella ostrina* collected in the Northern Hemisphere from lower latitude populations were more tolerant to heat stress than higher latitude populations as an adaptation to the heat events that they experienced in the south. Thus, the results obtained in this study suggest that embryos from the southern population (low-latitude) might possess genetic adaptations to tolerate warm temperatures; whilst embryos from the northern population (high-latitude) are

less adapted to such conditions. In fact, Mardones et al. (2020a) suggested that the aerobic response of encapsulated embryos of *O. erinaceus* from the same populations (i.e. Solent and Arcachon) are locally adapted to the temperature history of their natal environments. Embryos from the southern population showed higher respiration rates and metabolic adjustment to elevated temperatures; however, embryos from the northern population showed no metabolic adjustment, high capsular mortalities and limited acclimation to high temperatures (Mardones et al., 2020). In addition, a transplant study conducted on the same populations by Mardones et al. (2020b) demonstrated that there are adaptive cost to live under their natal conditions, resulting in trade-offs between reproductive trait. Embryos transferred from the southern to the northern location showed poor performance and high mortality under the northern conditions; which suggests that *O. erinaceus* is locally adapted to natal conditions. However, further analyses are necessary to elucidate whether the differences are genetically based.

Embryos from the northern and southern populations are living in environments that are very close to the upper tolerance limits. An increase of one or two degrees from the maximal temperatures that embryos normally experienced in the field (i.e. 18 and 20 °C for southern and northern population, respectively) increased the mortality or abnormalities during early development. Previous studies have reported that warm-adapted species may live at temperatures that are very close to their upper thermal limits in comparison with cold-adapted species (Reitzel et al., 2013; Tomanek, 2008; Zippay and Hofmann, 2010). For example, Somero et al. (2005) studied the thermal tolerance response of subtidal and intertidal porcelain crabs (genus *Petrolisthes*) collected from temperate and tropical locations. They found that warm-adapted crabs from the intertidal zone showed low capacity to acclimate to heat events than

subtidal species, because intertidal crabs are living very close to the upper thermal limits of the species. The upper thermal limit for heart function was 31.5 °C, and in the field they experienced temperatures between 31 and 32 °C; thus, an increase in temperature caused lethal effects. Because early stages of *O. erinaceus* from the southern and northern populations are living very close to their upper thermal limits, they might be at potential risk for extinction in scenarios of ocean warming (i.e. heat wave events).

At the end of the intracapsular development, temperature did not significantly affect the metamorphosis time in the southern population, swimming larvae spent between 24 and 48 hours in the water column. However, fifty percent of northern larvae at 16 and 18 °C delayed their metamorphosis for approximately 60 hours. These results appear unique for gastropods. For example, larvae of *Crepidula fornicata* (L) metamorphosed faster at 24 °C than at 18 °C temperatures (Pechenik, 1984). Pechenik (1984) concluded that delayed metamorphosis was related to developmental regimes: if larval development was fast, then metamorphosis would be reached sooner. Larvae of the congener *Crepidula plana* maintained at 29 °C were shown to be able to metamorphose more quickly than larvae maintained at low temperature 20°C (Zimmerman and Pechenik, 1991). The increased time to metamorphosis observed in northern larvae could be a result of high energetic demands and low larval nutritional reserves. It has been identified that exposure to high temperatures can produce high energetic cost for embryos (Cancino et al., 2011). Increases in metabolic rates could produce a drop in the larval energy reserves and produce deleterious effects on the metamorphosis. Indeed, 4% of the northern-hatched larvae at 18 °C were not able to metamorphose and died after 10 days as a swimming late-pediveliger, although their bioenergetic condition of the embryos was not

403 evaluated. Further study is required to understand this response of delays in metamorphosis
404 at high temperatures in *O. erinaceus.*

405 5. Conclusion

406 Global warming will negatively impact marine organisms, shifting species distribution and
407 promoting future extinctions. However the magnitude of the impact will depend on the
408 intraspecific adaptations of organisms to local environmental conditions. This study showed
409 that future warming will differently the thermal response of early stages of *O. erinaceus*
410 depending on the geographic location. Although both populations are living near to the upper
411 thermal tolerance limits, the northern population (Solent, UK) exhibited a warm-stenothermal
412 tolerance window between 14 -16 °C, with high mortalities at extreme temperatures. On the
413 contrary, the southern population (Arcachon, France) exhibited a wide, warm-eurythermal
414 tolerance between 12 and 18 °C, with abnormalities at both side of the thermal range.

415 In the context of climate warming, a possible outcome of this contrasting embryonic thermal
416 response -- besides the limited dispersal capability of *O. erinaceus* (i.e. direct developer) and
417 the potential for local adaptation-- could lead to shift the geographic distribution range
418 poleward. If southern populations adapted better to deal with warmer temperatures, then
419 they may geographically expand and fill the niche space left empty by local extinctions of more
420 northern populations, at the same time causing a decrease in the genetic variability and making
421 them more susceptible to localized mortality events. However, more studies are needed to
422 understand fully the potential impacts of climate change on the species' geographic
423 distribution at population-scale.

424

Declaration of competing interest

All authors have agreed on the submitted version of the manuscript, there is no conflict of interests. Neither the manuscript nor any part of it has been published previously, nor is it under consideration for publication elsewhere.

Acknowledgments

The authors would like thank R. Robinson, for helping with the setup of experiments at the NOCS. The authors also acknowledge the kind assistance of Professor Xavier de Montaudouin from Marine Station D'Arcachon for the collection of animals. This work was funded by the National Agency for Research and Development (ANID) /DOCTORADO BECAS CHILE/2015 – 72160099 and the University of Southampton.

References

Amio, M. (1963). A comparative embryology of marine gastropods with ecological emphasis. Journal of the Shimonoseki Collegue of Fisheries, 12, 229–353.

Anger, K., Thatje, S., Lovrich, G., & Calcagno, J. (2003). Larval and early juvenile development of *Paralomis granulosa* reared at different temperatures: Tolerance of cold and food limitation in a lithodid crab from high latitudes. Marine Ecology Progress Series, 253, 243–251.

446 Angilletta, M.J., Niewiarowski, P.H., & Navas, C.A. (2002). The evolution of thermal physiology
447 in ectotherms. Journal of Thermal Biology, 27, 249–268.

448 Burrows, M.T., Schoeman, D.S., Buckley, L.B., Moore, P., Poloczanska, E.S., Brander, K.M.,
449 Brown, C., Bruno, J.F., Duarte, C.M., Halpern, B.S., Holding, J., Kappel, C. V., Kiessling, W.,
450 O'Connor, M.I., Pandolfi, J.M., Parmesan, C., Schwing, F.B., Sydeman, W.J., & Richardson, A.J.
451 (2011). The pace of shifting climate in marine and terrestrial ecosystems. Science, 334, 652–
452 655.

453 Cancino, J.M., Gallardo, J.A., & Brante, A. (2011). The relationship between temperature,
454 oxygen condition and embryo encapsulation in the marine gastropod *Chorus giganteus*.
455 Journal of the Marine Biological Association of the United Kingdom, 91, 727–733.

456 Castañeda, L.E., Lardies, M.A., & Bozinovic, F. (2004). Adaptive latitudinal shifts in the thermal
457 physiology of a terrestrial isopod. Evolutionary Ecology Research, 6, 579–593.

458 Clemens-Seely, K., & Phillips, N.E. (2011). Effects of temperature on hatching time and
459 hatchling proportions in a poecilogonous population of *Haminoea zelandiae*. Biological
460 Bulletin, 221, 189–196.

461 Enricuso, O.B., Conaco, C., Sayco, S.L.G., Neo, M.L., & Cabaitan, P.C. (2019). Elevated seawater
462 temperatures affect embryonic and larval development in the giant clam *Tridacna gigas*
463 (Cardiidae: Tridacninae). Journal of Molluscan Studies, 85, 143–153.

464 Fernández, M., Pappalardo, P., & Jeno, K. (2006). The effects of temperature and oxygen
465 availability on intracapsular development of *Acanthina monodon* (Gastropoda: Muricidae).
466 Revista Chilena de Historia Natural, 79, 155–167.

467 Gaitan-Espitia, J.D., Bacigalupe, L.D., Opitz, T., Lagos, N.A., Osores, S., & Lardies, M.A. (2017).
468 Exploring physiological plasticity and local thermal adaptation in an intertidal crab along a
469 latitudinal cline. Journal of Thermal Biology, 68(PartA), 14-20.

470 Gibbs, P.E. (1996). Oviduct malformation as a sterilising effect of tributyltin (TBT) - induced
471 imposex in *Ocenebra erinacea* (Gastropoda : muricidae). Journal of Molluscan Studies, 662,
472 403–413.

473 Gleason, L.U., & Burton, R.S. (2013). Phenotypic evidence for local adaptation to heat stress in
474 the marine snail *Chlorostoma* (formerly *Tegula*) *funebralis*. Journal of Experimental Marine
475 Biology and Ecology, 448, 360–366.

476 Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S.,
477 Rodriguez, L.F., Tomanek, L., & Williams, S.L. (2006). The impacts of climate change in coastal
478 marine systems. Ecology Letters 9, 228–241.

479 Helmuth, B., Harley, C.D.G., Halpin, P.M., O'Donnell, M., Hofmann, G.E., & Blanchette, C.A.
480 (2002). Climate change and latitudinal patterns of intertidal thermal stress. Science 298, 1015–
481 1017.

482 Jansen, J.M., Pronker, A.E., Kube, S., Sokolowski, A., Sola, J.C., Marquiegui, M.A., Schiedek, D.,
483 Wendelaar Bonga, S., Wolowicz, M., & Hummel, H. (2007). Geographic and seasonal patterns
484 and limits on the adaptive response to temperature of European *Mytilus* spp. and *Macoma*
485 *balthica* populations. Oecologia, 154, 23–34.

486 Jones, S.J., Mieszkowska, N., & Wethey, D.S. (2009). Linking thermal tolerances and
487 biogeography: *Mytilus edulis* (L.) at its southern limit on the east coast of the United States.
488 The Biological Bulletin, 217, 73–85.

489 Kelly, M.W., Sanford, E., & Grosberg, R.K., (2012). Limited potential for adaptation to climate
490 change in a broadly distributed marine crustacean. Proceedings of the Royal Society Part B,
491 279, 349-356.

492 Kuo, E.S.L., & Sanford, E. (2009). Geographic variation in the upper thermal limits of an
493 intertidal snail: Implications for climate envelope models. Marine Ecology Progress Series, 388,
494 137–146.

495 Mardones, M.L., Fenberg, P.B., Thatje, S., & Hauton, C. (2020a) The role of temperature on the
496 aerobic response of encapsulated embryos of *Ocenebra erinaceus* (Neogastropoda,
497 Muricidae): A comparative study between two populations. Marine Environmental Research,
498 153: 104815.

499 Mardones, M.L., Fenberg, P.B., Thatje, S., & Hauton, C. (2020b) Intraspecific plasticity and
500 trans-generational adaptation of reproductive traits and early development in a temperate
501 marine gastropod. Marine Environmental Research, 161: 105123.

502 Martel, C., Guarini, J.M., Blanchard, G., Sauriau, P.G., Trichet, C., Robert, S., & Garcia-Meunier,
503 P. (2004). Invasion by the marine gastropod *Ocinebrellus inornatus* in France. III. Comparison
504 of biological traits with the resident species *Ocenebra erinacea*. Marine Biology 146, 93–102.

505 Pechenik, J.A. (1979). Role of encapsulation in Invertebrate Life Histories. The American
506 Naturalist, 114, 859–870.

507 Pechenik, J.A. (1984). The relationship between temperature, growth rate, and duration of
508 planktonic life for larvae of the gastropod *Crepidula fornicata* (L.). Journal of Experimental
509 Marine Biology and Ecology, 74, 241–257.

510 Pörtner, H.O. (2002). Climate variations and the physiological basis of temperature dependent
511 biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. Comparative
512 Biochemistry and Physiology - A Molecular and Integrative Physiology 132, 739–761.

513 Pörtner, H.O. (2010). Oxygen- and capacity-limitation of thermal tolerance: a matrix for
514 integrating climate-related stressor effects in marine ecosystems. Journal of Experimental
515 Biology 213, 881–893.

516 Przeslawski, R. (2004). A review of the effects of environmental stress on embryonic
517 development within intertidal gastropod egg masses. Molluscan Research, 24, 43–63.

518 Reitzel, A.M., Chu, T., Edquist, S., Genovese, C., Church, C., Tarrant, A.M., & Finnerty, J.R.
519 (2013). Physiological and developmental responses to temperature by the sea anemone
520 *Nematostella vectensis*. Marine Ecology Progress Series, 484, 115–130.

521 Roller, A., & Stickle, W.B. (1989). Temperature and salinity effects on the intracapsular
522 development , metabolic rates , and survival to hatching of *Thais haemastoma canaliculata*
523 (Gray) (Prosobranchia: Muricidae) under laboratory conditions. Journal of experimental
524 Marine Biology and Ecology, 125, 235–251.

525 Sanford, E., & Kelly, M.W. (2011). Local Adaptation in Marine Invertebrates. Annual Review of
526 Marine Science, 3, 509–535.

527 Sanford, E., Holzman, S.B., Haney, R.A., Rand, D.M., & Bertness, M.D. (2006). Larval tolerance,
528 gene flow, and the northern geographic range limit of fiddler crabs. Ecology 87, 2882–2894.

529 Schulte, P.M., Healy, T.M., & Fangue, N.A. (2011). Thermal Performance Curves , Phenotypic
530 Plasticity , and the Time Scales of Temperature Exposure, 51, 691–702.

531 Smith, K.E., Thatje, S., & Hauton, C. (2013). Thermal tolerance during early ontogeny in the
532 common whelk *Buccinum undatum* (Linnaeus 1785): Bioenergetics, nurse egg partitioning and
533 developmental success. Journal of Sea Research 79, 32–39.

534 Smith, K.E., Reed, A.J., & Thatje, S. (2015). Intracapsular development and dispersal
535 polymorphism in the predatory gastropod *Ocenebra erinaceus* (Linnaeus 1758). Helgoland
536 Marine Research 69, 249–258.

537 Somero, G.N. (2005). Linking biogeography to physiology: Evolutionary and acclimatory
538 adjustments of thermal limits. Frontiers in Zoology 2, 1.

539 Strathmann, R.R., & Chaffee, C. (1984). Constraints on egg masses. II. Effect of spacing, size,
540 and number of eggs on ventilation of masses of embryos in jelly, adherent groups, or thin-
541 walled capsules. Journal of Experimental Marine Biology and Ecology, 84, 85–93

542 Sunday, J.M., Bates, A.E., & Dulvy, N.K. (2011). Global analysis of thermal tolerance and latitude
543 in ectotherms. Proceedings of the Royal Society B: Biological Sciences, 278, 1823–1830.

544 Sunday, J.M., Pecl, G.T., Frusher, S., Hobday, A.J., Hill, N., Holbrook, N.J., Edgar, G.J., Stuart-
545 Smith, R., Barrett, N., Wernberg, T., Watson, R.A., Smale, D.A., Fulton, E.A., Slawinski, D., Feng,
546 M., Radford, B.T., Thompson, P.A., & Bates, A.E. (2015). Species traits and climate velocity
547 explain geographic range shifts in an ocean-warming hotspot. Ecology Letters, 18, 944–953.

548 Tomanek, L. (2008). The Importance of Physiological Limits in Determining Biogeographical
549 Range Shifts due to Global Climate Change: The Heat-Shock Response. Physiological and
550 Biochemical Zoology, 81, 709–717.

551 Yu, D., Zhang, Z., Shen, Z., Zhang, C., & Liu, H. (2018). Regional differences in thermal
552 adaptation of a cold-water fish *Rhynchocypris oxycephalus* revealed by thermal tolerance and
553 transcriptomic responses. Scientific Reports, 8, 1–11.

554 Zimmerman, K.M., & Pechenik, J.A. (1991). How Do Temperature and Salinity Affect Relative
555 Rates of Growth, Morphological-Differentiation, and Time to Metamorphic Competence in
556 Larvae of the Marine Gastropod *Crepidula plana*. Biological Bulletin, 180, 372–386.

557 Zippay, M.L., & Hofmann, G.E. (2010). Physiological tolerances across latitudes: Thermal
558 sensitivity of larval marine snails (*Nucella* spp.). Marine Biology, 157, 707–714.

559

560

561

562

563