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1 **Maternal and cohort effects modulate offspring responses to multiple stressors**

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ABSTRACT

24 Current concerns about climate change have led to intensive research attempting to
25 understand how climate-driven stressors affect the performance of organisms, in particular
26 the offspring of many invertebrates and fish. Although stressors are likely to act on several
27 stages of the life cycle, little is known about their action across life phases, for instance
28 how multiple stressors experienced simultaneously in the maternal environment can
29 modulate the responses to the same stressors operating in the offspring environment. Here,
30 we study how performance of offspring of a marine invertebrate (shore crab *Carcinus*
31 *maenas*) changes in response to two stressors (temperature and salinity) experienced during
32 embryogenesis in brooding mothers from different seasons. On average, offspring
33 responses were antagonistic: high temperature mitigated the negative effects of low salinity
34 on survival. However, the magnitude of the response was modulated by the temperature
35 and salinity conditions experienced by egg-carrying mothers. Performance also varied
36 among cohorts, perhaps reflecting genetic variation, and/or maternal conditions prior to
37 embryogenesis. This study contributes towards the understanding of how anthropogenic
38 modification of the maternal environment drives offspring performance in brooders.

39 **Keywords:** climate change, maternal effects, multiple stressors, offspring performance,
40 salinity, temperature

INTRODUCTION

41

42 Current and future estimates of climate-related changes in the marine environment have
43 emphasised the necessity to understand the importance of multiple-driver (or stressor)
44 effects on organisms, populations, communities, and ecosystems^{1,2,3,4}. The main issue
45 being that climate change results in multivariate modifications in marine habitats with
46 environmental variables reaching values that are near, or beyond, normal levels of
47 variation. In such cases, environmental drivers of biotic responses may become stressors
48 because they elicit a stress response, which may manifest as reductions in performance of
49 individuals (e.g. lower survival or prolonged developmental periods). Understanding the
50 cumulative impact of multiple drivers is considered to be one of the most pressing research
51 goals in environmental sciences⁵.

52 We are beginning to appreciate that the effects of multiple drivers cannot be predicted
53 from studies on single environmental variables due to the frequent detection of interactive
54 effects^{6,7,8}. Such interactions can be antagonistic or synergistic^{8,9,10}; depending on whether
55 the presence of a driver exacerbates or mitigates the effect of a second driver. While
56 synergistic interaction means that the effects are larger than the sum of the effects elicited
57 by each single environmental driver, antagonistic interaction refers to effects that are less
58 than the sum. The latter suggests some capacity of organisms to tolerate environmental
59 change. The prevalence of each interaction is poorly understood as some studies have
60 reported synergistic interactions^{4,7} while others have reported additive effects, or
61 antagonistic interactions^{11,12,13}. Moreover, interactive effects of multiple stressors appear
62 to vary across taxa, developmental stages and trophic levels.

63 An area that deserves attention concerns scenarios where environmental conditions
64 fluctuate over time⁴. For instance, in coastal-estuarine habitats, salinity and temperature, as
65 well as other environmental drivers can vary, especially in regions of freshwater influence,
66 where spatial patterns in salinity are driven by estuarine or river plumes¹⁴. Temperature
67 and salinity can also co-vary with season^{15,16,17}. In summer, coastal-estuarine waters of
68 lower salinity are usually warmer than coastal shelf seawaters. Natural variations
69 associated with tidal cycles or freshwater runoff, or the fact that many organisms migrate
70 across coastal gradients, means that individuals will experience periods of lower salinity
71 coinciding with higher temperatures. Moreover, the covariation between temperature and
72 salinity, as experienced by organisms in the summer, may reverse in the winter (brackish
73 waters often being cooler than seawater^{16,18}) and may be weaker in spring/autumn or during
74 long periods of rainfall due to cooler allochthonous inputs of freshwater from land.

75 Environmental fluctuation encountered during the maternal-offspring transition (e.g.
76 hatching and larval release) can be critical. In brooding species, offspring are often released
77 into a new environment¹⁹ that contrasts with the conditions experienced in the egg mass
78 during embryogenesis. Offspring appear to be particularly sensitive to genetic or
79 developmental malfunctions²⁰ and environmental change may trigger a number of adaptive
80 or non-adaptive phenotypic responses^{21,22,23}. Responses occurring at such time are
81 dominated by maternal effects i.e., effects of the maternal environment or phenotype on
82 offspring phenotype and performance^{24,25,26}. At the evolutionary scale, theory predicts that
83 maternal effects evolve under sudden environmental shifts or changes consistent with those
84 of climate change²⁷; in addition maternal effects are expected to evolve in seasonal
85 environments²⁸ such as temperate estuaries. Maternal effects, driven by environmental

86 change, may occur before fertilization (prezygotic effects²⁶, e.g. variation in allocation of
87 reserves into eggs), during embryogenesis (post-zygotic effects^{29,30,31,32}) or after hatching
88 and offspring release (post-natal maternal effects). Few studies, however, have managed to
89 study these complexities and assessed the relative importance of changes in multiple
90 environmental drivers before or after fertilisation in brooding marine species. Yet, such
91 studies are needed in order to obtain a more realistic picture of how organisms will cope
92 with climate driven modifications of the natural habitat. If maternal effects modulate
93 offspring responses, then responses obtained from studies ignoring such effects either over-
94 or underestimate the offspring capacity to cope with climate change.

95 Here, we evaluate the importance of maternal effects in modifying responses to
96 temperature and salinity in early larval stages of the estuarine-coastal crab *Carcinus*
97 *maenas*. *C. maenas* is an euryhaline crab that is endemic to northern Europe^{33,34,35} but
98 considered a global invader. Larvae of *C. maenas* exhibit an antagonistic response to low
99 salinity and increased temperature, (“thermal mitigation of low salinity stress”: TMLS; Fig.
100 1) whereby negative effects of low salinity on survival and developmental time are
101 mitigated at high temperatures³⁶. The most likely underpinning mechanism is an increase
102 in osmoregulatory capacity at higher temperatures: thus, TMLS may be a consequence of
103 this physiological plasticity. The TMLS is found in other coastal species and it is relevant
104 in the light of climate change in that (moderate) warming may favour expansion towards
105 coastal areas characterised by moderately low salinities³⁶. However, the same study also
106 found that responses vary among larvae from different females³⁶; which may be driven by
107 variability in the maternal environment, for instance by the temperature and salinity
108 experienced by females and embryos. Theoretically, salinity and temperature may alter

109 embryonic developmental processes and hence modify larval performance in many
110 possible ways. For example, suboptimal conditions in the maternal habitat (where embryos
111 develop) may weaken or pre-empt the development of antagonistic responses (Fig. 1, Pre-
112 emption) or induce synergistic stress responses (Fig. 1, Induction). In both cases, the
113 assessment of offspring responses to stressors, without considering such maternal effects,
114 will over-estimate the capacity of offspring to cope with climate driven change.

115 In order to establish which scenario from Figure 1 prevails in the shore crab, we studied
116 the role of the maternal post-zygotic environment in modifying larval performance in
117 response to temperature and salinity. Our approach was to examine the effects of salinity
118 and temperature experienced by embryos during brooding, on the survival and
119 developmental time of resultant first stage larvae, which were also exposed to the same
120 stressors in a factorial design. In addition, we performed the experiments with larvae from
121 females producing eggs at different times of the year (autumn vs. early summer), in order
122 to determine if post-zygotic responses are consistent or if they vary among cohorts.
123 Differences in responses among larvae from different cohorts (but otherwise kept under
124 similar temperature-salinity conditions over both the embryonic and larval phases) should
125 be driven by genetic differences among broods or the influence of prezygotic maternal
126 effects²³. Ultimately, we were interested in obtaining a more general picture on how
127 offspring response may be modulated by maternal effects and how such modulation may
128 vary among cohort of females.

129

130 ***FIGURE 1***

131

MATERIALS AND METHODS

Animal husbandry, larval rearing and experimental design

Berried females of *Carcinus maenas* were collected in the Menai Strait (North Wales, UK) in autumn (October-November) and early summer (May-June) and transferred to marine aquaria in the School of Ocean Sciences, Bangor University (UK). On the day of collection, embryos were staged and females were distributed in the experimental treatments. Females carrying eggs at early stages of development (i.e. at the initiation of the formation of the embryo) were distributed at random into four treatments consisting of two temperatures (15°C and 18°C) and two salinities (diluted seawater: 25 PSU and seawater: 35 PSU; salinity is expressed in PSU, equivalent to ppt, following standard convention in oceanography). Those treatment combinations represent suboptimal (moderate osmotic and thermal stress) and optimal conditions (Fig. S1); preliminary experiments, using females from the same population, revealed that hatching of viable larvae was still possible at 15°C and a salinity of 25 PSU. Females (carapace width: average=50.1 ± SD=8.7 mm) were randomly distributed among the treatments. We ran preliminary correlation analyses with female size as a covariate, but we did not find any relationship. Therefore, we did not consider female body size in the subsequent analyses.

Females with embryos at the earliest possible developmental stage were used to ensure that the embryos experienced different temperature and salinity combinations for a minimum of two weeks (Table S1) and that they were exposed at the time of the formation of the first larval stage (zoea I). This applied to all females from the autumn cohort, as well as the majority of the early summer cohort. For the latter, we had to discard a large number of berried females because of a parasitic infection within the egg mass. This led to only

155 one surviving female being allocated to a salinity of 35 PSU and temperature of 18°C.
156 Three females carrying fully formed embryonic zoea were also included, as this treatment
157 combination is the same as natural summer conditions. These females were exposed to the
158 treatment for 4 days before hatching (Table S1). Figure S2 shows that, for this group, there
159 is little variation in survival among broods.

160 The resulting larvae from the brooding females were held in six different combinations
161 of temperature and salinity (15, 18 and 24°C, and 20 and 35 PSU) in a fully factorial design,
162 representing the offspring environment. The salinities were chosen to reflect the higher
163 tolerance to lower salinity of larvae (20 PSU) compared to the embryos (25 PSU). In
164 addition, we added a higher temperature to test effects of extreme temperatures on the
165 larvae (24°C). In total, this gave 24 different combinations of embryonic and larval
166 temperature and salinity conditions (Fig. S1). Each berried female occupied an individual
167 aquarium (volume: 3L) supplied with fully aerated seawater. Aquaria were placed in two
168 holding tanks (1.5m length x 1.0m width x 0.5m height) thermostatically controlled at the
169 desired temperature (15 or 18°C, respectively) by heating/cooling units. Each aquarium
170 was supplied with natural seawater or appropriately diluted seawater for low salinities (see
171 below for details). Water was taken from the Menai Strait, which was filtered (0.2µm),
172 UV-treated, and aerated prior to use (at a Salinity of 34 PSU and temperature of 15°C, pH
173 = 8.00, $A_T = 2286 \mu\text{mol kg}^{-1}$, $\text{DIC} = 2140 \mu\text{mol kg}^{-1}$, $p\text{CO}_2 = 599 \mu\text{atm}$. N.M. Whiteley
174 unpublished observations). Twice a week, and two hours prior to the water change, females
175 were offered mussels as food. Larvae were held in 100 ml filtered, UV treated, aerated
176 seawater or appropriately diluted seawater for low salinities in open necked shallow
177 beakers and placed within temperature-controlled incubators (LMS, series 4, UK). Twenty-

178 four hours prior to each water change, seawater dilution (for the lower salinity treatments)
179 was achieved in separate holding tanks using a conductivity meter (WTW 315i) to
180 determine salinity in natural seawater mixed with appropriate quantities of de-chlorinated
181 tap water. Both females and larvae were maintained with a photoperiod 12:12 (light:dark
182 hours). During the embryonic and larval exposures, temperature and salinity were
183 measured daily while the water was replaced. Readings were stable throughout the
184 incubations (variation less than a salinity of 0.1PSU or 0.1°C).

185 Larvae hatched from each female were assigned randomly to each of six treatments, in
186 five replicates (10 freshly hatched Zoea I each), each one consisting of a 100 ml beaker; all
187 females produced sufficient larvae for experiments (*C. maenas* fecundity ca. 180.000
188 embryos per clutch³⁷). Larval rearing followed standard methods^{36,38}: seawater and food
189 (*Artemia* sp. *ad libitum*: 5 individuals ml⁻¹) were changed daily and dead larvae were
190 recorded and discarded. The experiment finished when all larvae died or moulted to Zoea I
191 II. We quantified larval performance as survival (i.e. the proportion of initial Zoea I
192 reaching the Zoea II) and the duration of development (i.e. the time of development from
193 hatching until moult to Zoea II).

194

Data analysis:

195 Larval performance was evaluated as survival and duration of development of the first
196 zoeal stage. It is at this stage when maternal effects are likely to be more important; in
197 addition, the TMLS is well developed during the first zoeal stage³⁶. Survival data
198 (proportion) were first adjusted using the equation $p' = [p(n-1)/n+0.5]/n$, ($n=10$
199 individuals) and then analysed after logistic (= logit) transformation³⁹, following Griffen
200 *et al.*⁸. For survival, we applied a five-way factorial model containing embryonic salinity

201 (E_S), embryonic temperature (E_T), larval salinity (L_S), larval temperature (L_T) and season
202 (S). We use the term “embryonic” instead of “maternal” in order to emphasise our focus
203 on post-zygotic maternal effects. There was an additional factor, female (F) which
204 represents the within-cohort variation in the responses; i.e. variation in responses of
205 individuals originating from different females and experiencing the same environments as
206 embryos and larvae. We did not separate the embryos from the mothers because embryonic
207 development and hatching are impaired when embryos are isolated from the mother⁴⁰.
208 Thus, the factor female was nested in the interaction between embryonic temperature,
209 embryonic salinity and season, because each female belonged to a season and its respective
210 embryos experienced a specific salinity-temperature combination. The between-cohort
211 effect is captured by the term (S) and represents differences in the responses among
212 individuals originating from females belonging to different cohorts and experiencing the
213 same environments as embryos and larvae.

214 The duration of development was analysed using the data corresponding to the larvae
215 reared in seawater because we had high mortality rates at a lower salinity of 20 PSU (see
216 Results). The starting model was reduced to a four-way factorial model (the factor “larval
217 salinity”, L_S, was dropped), keeping female (F) as a random factor. This model still enabled
218 us to test up to fourth order interactive effects (e.g. E_T:E_S:L_T:S). Statistical analyses were
219 run (separately) on the raw and log-transformed data in order to determine if interactive
220 responses observed on the raw data (interaction term retained during model selection)
221 reflected proportional effects (the same term is not retained for log transformed data).

222 Statistical analyses were carried out through linear mixed model effects⁴¹, in R⁴² using
223 the package nlme⁴³. In addition to the terms in the model, we controlled for variance

224 heterogeneity among replicates (using the VarIdent constructor function⁴³). Although our
225 design was fully replicated, our attempts at fitting the full model led to situations of a
226 singular matrix, suggesting that some components were not estimated⁴⁴. When this
227 occurred, we followed procedures outlined by Bolker *et al.*⁴⁴ and reduced the complexity
228 of the starting model. We used a combination of model selection (based on AIC) and
229 hypothesis testing approaches as follows. First, model selection was applied through the
230 backwards approach (i.e. starting with the full model) and then ranking models through
231 Akaike information criteria (AIC), detecting differences between the model with the lowest
232 AIC and any other model (Δ AIC). When the simplest model had the lowest AIC, that model
233 was selected; if Δ AIC > 3, the model with lower AIC was selected irrespective of
234 differences in complexity. Hypothesis testing (likelihood ratio tests) was applied only when
235 Δ AIC < 3, and the most complex model had the lower AIC. When models differed
236 significantly ($p < 0.05$) the one with lower AIC was selected; in the opposite situation, the
237 principle of parsimony was applied and the model with lower number of parameters was
238 selected. Model selection was applied in two steps, (1) on the random structure (i.e.
239 variance heterogeneity and effects of female of origin, interacting with larval salinity and
240 temperature) using the restricted maximum likelihood method (REML). Then, (2) on the
241 fixed structure (i.e. effects of season, embryonic and larval salinity and temperature)
242 through maximum likelihood (ML).

243

RESULTS

244

Survival to Zoea II

245 Larval survival showed complex responses to changes in temperature and salinity in the
246 offspring environment (Table S2). Larval survival showed an antagonistic response, called
247 thermal mitigation of low salinity stress (TMLS³⁶). Survival was lower at low salinity, but
248 improved at 18°C or 24°C, compared with survival at 15°C (Fig. 2 a-c). Plots of larval
249 survival per brooding female showed that survival at low salinity peaked either at 18°C, or
250 at 24°C, depending on female (Fig. S3). Consistent with the TMLS response, temperature
251 and salinity interacted to influence survival which differed from the multiplicative model
252 of survival. For example, taking 15°C and a salinity of 35 PSU as the control condition
253 (average survival = 76%) the expected independent effect of each variable is the product
254 of the survival probability observed at increased temperature, but optimal salinity (=66%),
255 and that observed at reduced salinity, but optimal temperature (=3%). The expected effect
256 under the hypothesis of independence is 2% (= 0.66 x 0.03), which is eight times lower
257 than observed survival (17%) at 24°C and salinity of 20 PSU and is consistent with the
258 TMLS.

259

260 *FIGURE 2*

261

262 The TMLS response was modulated by embryonic salinity (Fig. 2a, Table S2: E_S:L_S:L_T:
263 LR=15.10, p<0.001) and embryonic temperature (Fig. 2b, Table S2: E_T:L_S:L_T: LR=8.98,
264 p=0.011). The mitigation effect was weaker when embryos were kept at low salinity (= 25

265 PSU). In larvae exposed to salinity of 20 PSU and 24°C, average survival was only ~7%
266 when larvae hatched from embryos kept at salinity of 25 PSU, while average survival was
267 ~28% when larvae hatched from embryos kept at a salinity of 35 PSU, which was a 4-fold
268 difference. In addition, the mitigation effect was weaker when embryos were kept at 15°C.
269 For instance, larvae exposed to a salinity of 20 PSU and temperature of 24°C had a survival
270 of ~10% when hatched from embryos kept at 15°C, but under the same larval conditions,
271 survival was ~21% when embryos were previously kept at 18°C marking a two-fold
272 increase in survival. In summary, low temperature (15°C) or low salinity (25 PSU)
273 experienced at the embryonic stage weakened the thermal mitigation of low salinity stress.

274 The magnitude of TMLS varied between cohorts and among females of the same cohort.
275 Larvae hatching from females of the autumn cohort showed stronger TMLS than those of
276 the spring-summer cohort (Fig. 2c; Table S2: S:L_S:L_T: LR = 8.98, p=0.011). When reared
277 at a salinity of 20 PSU at 24°C, larvae from the spring-summer cohort showed an average
278 survival of 2.4%, while those of the autumn cohort showed an average survival of 26% (i.e.
279 10-fold increase in survival). In addition, the larvae exposed to a salinity of 35 PSU from
280 the autumn cohort had on average, higher survival than those of the spring-summer cohort
281 (Fig. 2c). Within cohorts, female effects (retained in the random structure of the model)
282 consisted mainly in variations in the strength of the TMLS (Fig S3a): this is shown as an
283 important variation in survival at low salinity when larvae were exposed to 24°C, observed
284 more clearly in the autumn cohort. At high salinity (Fig S3b), the female effect appears to
285 occur irrespective of larval temperature conditions.

286 The cohorts also differed in terms of the sequential effects of embryonic salinity and
287 larval salinity (Fig. 2d; Table S2: S:E_S:L_S: LR = 4.89, p =0.027). For the autumn cohort,

288 high embryonic salinity ameliorated the effect of low larval salinity on survival, but such
289 an effect did not occur in the spring-summer cohort. For the autumn cohort, the survival of
290 larvae hatching from embryos kept at a salinity of 25 PSU was ~8% while those hatching
291 from embryos kept at a salinity of 35 PSU had a survival of ~24% (i.e. a three-fold
292 increase). There were also significant differences in survival between cohorts in response
293 to embryonic salinity and temperature (Table S2: S:ES:ET: LR=5.80, p=0.016), but these
294 effects were weak (Fig. S4a) and not detected by post-hoc tests. The same was true for the
295 effect of the embryonic salinity and larval temperature on survival (Table S2: S:ES:LT: LR
296 = 7.32, p=0.026; Fig. S4b) and in both cases survival was generally higher in larvae from
297 the autumn cohort.

298 **Development time to Zoea II**

299 The duration of development had a complex fourth order interactive response when
300 analyses were based on the raw data (Fig. 3; S:ES:ET:LT: LR=7.79, p=0.02; model selection
301 summarised in Table S3). Duration of development was driven mainly by larval
302 temperature (L_T); as expected, larvae developed faster at higher temperatures with average
303 differences of 2 to 4 days between larvae reared at 24°C and 15°C (effects of larval salinity,
304 were not tested due to high mortality at low salinity).

305

306 ***FIGURE 3***

307

308 In the spring-summer cohort (Fig. 3a), low embryonic salinity and temperature induced
309 a stress response in larvae reared at 15°C: development of such larvae was ca. 2 days longer

310 than when exposed to other embryonic conditions (at the same larval temperature). Such a
311 stress response was also absent from the autumn cohort (Fig. 3b).

312 After logarithmic transformation, the four-way interaction was dropped from the model
313 but the interactive effect of embryonic salinity and temperature was retained (S:Es:Et:
314 LR=9.54, p=0.002; model selection summarised in Table S4). In both seasons, the effect
315 of temperature on duration of development was stronger in the spring-summer cohort at
316 low embryonic salinities. Duration of development was longer in the spring-summer than
317 in the autumn cohort. Larval temperature had a strong effect which varied with cohorts
318 (S:L_T: LR=10.16, p=0.006): the duration of development at 24°C was about 70% of that at
319 15°C for the autumn cohort and 60% for the spring-summer cohort.

320

321

DISCUSSION

322 We found that post-zygotic maternal effects can modulate performance of offspring of
323 shore crab *C. maenas* in response to salinity and temperature, and that such responses vary
324 among seasonal cohorts. The main response was observed in terms of survival, where we
325 found further evidence for a thermal mitigation of low salinity stress (TMLS³⁶), now
326 extended to the local population of the Irish Sea. Developmental duration showed a
327 response, consistent with TMLS: duration was extended in larvae reared at low
328 temperatures when such larvae hatched from embryos reared at low temperature and low
329 salinity. Both responses are manifested when larvae are reared at low temperatures. At least
330 the TMLS may be based on an increase in osmoregulatory capacity of the first zoeal stage³⁴
331 at high temperatures: osmoregulation is usually enhanced at high temperatures^{45,46} which
332 increase the capacity of mitochondria to produce ATP⁴⁷ and the ability to repair damage.

333 The TMLS response of the larvae was modulated by salinity and temperature
334 experienced during embryogenesis. Reduced salinity and temperature did not fully pre-
335 empt (see Fig. 1) but weakened the capacity of the larvae to exhibit TMLS. The fact that a
336 strong TMLS was observed after high embryonic temperature may have important
337 implications in that larvae will be more capable of surviving decreased salinities in a
338 warming world due to the combined action of temperature operating on both the embryonic
339 and larval phases. Because, TMLS is a common feature at least in coastal crustaceans^{48,49,50},
340 an important question is whether moderate warming in the maternal habitat (at the time of
341 embryogenesis), may further increase the magnitude of TMLS and favour the invasion of
342 those near-shore habitats characterised by moderately low salinities. Moderately increased
343 temperature can lead to adaptive transgenerational plasticity, although extreme
344 temperatures can disrupt adaptive plasticity⁵¹.

345 An important question concerns the mechanisms underpinning the maternal modulation
346 of larval responses. In estuarine species, there is a strong modulation of salinity tolerance
347 through acclimatory responses^{30,49}, (i.e. a form of developmental plasticity whereby the
348 larval tolerance to low salinity increases if embryos are also exposed to low salinity), based
349 on an increase in the osmoregulatory capacity⁵². However, we did not find evidence of
350 embryonic acclimation to low salinities in *C. maenas*; by contrast, low salinity experienced
351 during embryogenesis weakened the TMLS. Perhaps, exposure to low salinity depletes
352 energy reserves during embryogenesis^{53,54} with a resulting decrease in larval reserves,
353 survival and developmental rate. By contrast, exposure of embryos to optimal temperatures
354 can result in wider larval tolerances to temperature and salinity⁵⁵ which may accelerate the
355 formation of osmoregulatory tissue.

356 How does the weakening of TMLS or the induction of a stress response (observed as
357 extended duration of development) relate to the various known maternal effects²⁵? Our
358 results need to be interpreted in the context of ontogenetic shifts in physiological tolerance
359 because preliminary experiments showed that ovigerous females incubated at a salinity of
360 20 PSU rejected their eggs (G. Torres unpublished observations), while larvae were able
361 to tolerate this salinity. For such reason we cannot establish correspondences with, for
362 instance, the concept of adaptive matching, whereby the best offspring performance occurs
363 when the maternal and offspring environments coincide²⁵. Ontogenetic shifts in
364 physiological tolerance should be widespread in brooding species where embryogenesis
365 takes place at habitat conditions that differ considerably from those experienced by
366 larvae^{30,38,49}, or where embryogenesis and larval development take place over different
367 seasons^{56,57}.

368 Another important result concerned the variation in the magnitude of TMLS and in the
369 duration of development in larvae hatched from different cohorts (= broods produced in
370 different seasons). Inter-cohort variation in response to climate driven stressors is arising
371 as a major feature and can appear at several time scales (e.g. bi-weekly⁵⁸; seasonal⁵¹; this
372 study; among years^{36,58}). Inter-cohort variation in the performance of organisms is
373 important because they can stabilise or de-stabilise the populations dynamics^{59,60}. For *C.*
374 *maenas*, we do not have sufficient information about the structure of population and thus
375 we can only speculate on how inter-cohort variation may affect the dynamics. Higher
376 performance in larvae resulting from autumn embryos may contribute to recruitment by
377 buffering offspring from potential suboptimal (winter-spring) conditions; this would be
378 similar to a case where a few individuals of high quality secure resources and avoid a

379 population crash⁶⁰. Larvae resulting from autumn embryos may also have a
380 disproportionate contribution to recruitment (as compared with larvae from the spring-
381 summer cohorts) if conditions are optimal. Disproportionate contributions to the
382 population-biomass by embryos produced in winter (as compared to “summer embryos”)
383 appear to occur in natural populations of another coastal crustacean (brown shrimp
384 *Crangon crangon*) in the North Sea⁶¹. Such embryos hatch into larvae that have higher
385 tolerance to food limitation than those of summer embryos⁶², but the contribution of winter
386 embryos to the population appears to occur through an additional number of factors (e.g. a
387 seasonal pattern of mortality rate). In principle, inter-cohort variation may reflect genetic
388 variation as well as pre- and post-zygotic maternal effects; plasticity may arise because, for
389 instance, the temperature experienced by parents and embryos in a summer-autumn cohort
390 will be higher than that experienced by the spring cohort (e.g. see⁵¹). In our case,
391 differences in performance among cohorts were detected among individuals hatching from
392 embryos kept at the same temperature and salinity. Given that the post-zygotic conditions
393 were kept constant, inter-cohort differences must reflect genetic variability or prezygotic
394 effects. In addition, variation in the responses should also reflect longer term
395 transgenerational plasticity, for instance grand-parental effects⁶³, which can only be teased
396 apart by experiments running over several generations.

397 Previous studies have pointed to the necessity to understand the role of within and
398 transgenerational phenotypic plasticity and genetic variation^{21,27,64} in determining the
399 capacity of organisms to respond to climate change. By focusing on an invasive marine
400 brooder, this work highlights the importance of post-zygotic effects (see also^{30,51,58}) as
401 modulators of larval responses to multiple environmental drivers, which may be relevant

402 to understand how brooders cope with climate change. Furthermore, this study highlights
403 the need of cross-habitat conservation programs in species undergoing habitat shifts, as
404 conditions in the maternal habitat determine the provision for the offspring with the
405 physiological machinery to tolerate environmental stressors in the larval habitat.

406

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415 parliament and of the Council of 22nd September 2010 on the protection of animals used
416 for scientific purposes.

417 **Data accessibility:** The datasets used and/or analysed during the current study are
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419 **Author's contributions:** GT and LG conceived this study and designed the
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- 601

602

FIGURE LEGENDS

603 **Figure 1.** Scenarios of maternal modulation of offspring performance. In an optimal
604 maternal environment (M_E : optimal; left panel) larvae exhibit an antagonistic response
605 (TMLS) whereby reductions in performance, resulting from low salinity (L_S), are mitigated
606 at moderately high temperatures (L_T). A suboptimal maternal environment (M_E :
607 suboptimal) either pre-empts (middle panel) larvae to exhibit TMLS (i.e. responses to
608 salinity are independent of temperature) or induces (right panel) a synergistic response
609 (high temperature exacerbates the stressful effects of low salinity).

610

611 **Figure 2.** Survival of *Carcinus maenas* larvae to Zoea II. (a) Interaction between
612 embryonic salinity (E_S), larval temperature (L_T) and larval salinity (L_S). (b) Interaction
613 between embryonic temperature (E_T), larval temperature and larval salinity. (c) Interaction
614 between season (S), larval temperature and larval salinity. (d) Interaction between season,
615 embryonic salinity and larval salinity. Different letters indicate significant differences
616 among the specific treatment combinations plotted within each panel. Values shown as
617 mean \pm standard error among larvae hatched from n (see Table S1) different females.

618

619 **Figure 3.** Development duration of *Carcinus maenas* larvae to Zoea II in seawater (i.e.
620 $L_S=35$). Four-way interaction between season (S), embryonic temperature (E_T) and
621 salinity (E_S), and larval temperature (L_T). (a) Spring-summer cohort. (b) Autumn cohort.
622 In (a) asterisk indicates significant differences among larvae exposed to 15°C. In (b)
623 different numbers beside the symbols indicate significant differences between larval

624 temperatures. Values shown as mean \pm standard error among larvae hatched from n (see
625 Table S1) different females.