Contrasting offspring responses to variation in salinity and temperature among populations of a coastal crab: A maladaptive ecological surprise?

Šargač, Z. ; Gimenez, Luis; Harzsch, Steffen; Krieger, Jakob; Fjordside, Kasper; Torres, Gabriela

Marine Ecology Progress Series

DOI:
10.3354/meps13851

Published: 28/10/2021

Peer reviewed version

Contrasting offspring responses to variation in salinity and temperature among coastal populations: a maladaptive ecological surprise?

Zoran Šargač[1], Luis Giménez[2,3], Steffen Harzsch[1], Jakob Krieger[1], Kasper Fjordside[4], Gabriela Torres[2]*

[1]University of Greifswald, Zoological Institute and Museum, Department of Cytology and Evolutionary Biology, 17489 Greifswald, Germany.
[2]Biologische Anstalt Helgoland, Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, 27498 Helgoland, Germany.
[3]School of Ocean Sciences, Bangor University, LL59 5AB Menai Bridge, United Kingdom.
[4]University of Southern Denmark, Department of Biology, Marine Biological Research Centre, 5300 Kerteminde, Denmark.

***Corresponding author**
Dr. Gabriela Torres
Ostkaje 1118
27498 Helgoland
Email: Gabriela.Torres@awi.de
https://orcid.org/0000-0002-4064-0585

Abstract

Current understanding of species capacities to respond to climate change is limited by the amount of information available about intraspecific variation in the responses. Therefore, we quantified between- and within- population variation in larval performance (survival, development, and growth to metamorphosis) of the shore crab *Carcinus maenas* in response to key environmental drivers (temperature, salinity) in two populations from regions with contrasting salinities (32-33PSU: Helgoland-North Sea; 16-20 PSU: Kerteminde-Baltic Sea). We also accounted for the effect<s> of salinity experienced during embryogenesis, which differs between populations. We found contrasting patterns between populations and embryonic salinity conditions. In the Helgoland population, we observed a strong Thermal Mitigation of Low Salinity stress (TMLS) for all performance indicators, when embryos were kept in seawater. The negative effects of low salinity on survival were mitigated at increased temperatures; only at high temperatures, larvae exposed to low salinity were able to sustain high growth rates and reduced developmental time, thereby metamorphosing with comparable levels of carbon and nitrogen to those reared in seawater. By contrast, larvae from the Kerteminde population showed a detrimental effect of low salinity, consistent with a maladaptive response and a weak TMLS. Low salinity experienced during embryogenesis pre-empted the development of TMLS in both populations, and reduced survival for the Kerteminde population, which is a maladaptive response. Our study emphasises the importance of evaluating species responses to variation in temperature and salinity across populations; the existence of maladaptive responses and the importance of the maternal habitat should not be underestimated.

Keywords: interpopulation variation, post-zygotic effects, larval performance, environmental drivers, *Carcinus maenas*.

1. Introduction

Fluctuations of Earth's climate and global warming have major effects on biological systems at several levels of organisation (Burrows et al. 2011, Poloczanska et al. 2013, Boersma et al. 2016, Boyd et al. 2018), and are already affecting species' physiology, distribution, and community composition (Perry et al. 2005, Somero 2010, Burrows et al. 2011, Poloczanska et al. 2013, Reusch 2013). As climate changes, organisms need to cope with variation in many different environmental variables or stressors (thereafter called "drivers"). The interplay between two or more drivers often has non-independent effects with one enhancing (as a synergistic effect) or weakening (antagonistic effect) the effect of another driver, beyond the additive effect expected from the action of each driver considered in isolation (Folt et al 1999, Crain et al. 2008, Todgham & Stillman 2013, Piggott et al. 2015, Orr et al. 2020, Tekin et al. 2020). Both synergistic and antagonistic effects may lead to various outcomes for particular species including the collapse of a biotic system (Breitburg et al. 1998) and potential adaptive response to multiple environmental changes (Sinclair et al. 2013). In addition, we expect that interactions between multiple drivers are responsible for the fate of many coastal species because coastal habitats are strongly influenced by climate change (Hiddink et al. 2015, Holt et al. 2016, Robins et al. 2016, Tinker et al. 2016). For instance, temperatures in the North and Baltic Seas are expected to increase, while salinity in the Baltic is predicted to decrease (BACC I 2008, Neumann 2010, Meier et al. 2012, Andersson 2015), and change is already happening in those seas (Wiltshire et al. 2010, Burrows et al. 2011, Boersma et al. 2016). Salinity and temperature regimes are crucial drivers for coastal biota (Hänninen et al. 2000, Telesh et al. 2013), affecting performance and fitness (Somero 2005, 2010, Ko et al. 2014). In general, coastal, estuarine, and intertidal species have adapted to cope with large ranges of temperature and salinity but they are often at their physiological limits (Stillman & Somero 1996, 2000, Browne & Wanigasekera 2000).

Recently, there has been an interest in the importance of intraspecific trait variation as an important biological feature in understanding responses to climate change (highlighted by Moran et al. 2016). Intraspecific trait variation can occur at several spatial and temporal scales (Violle et al. 2014) and can shape species distribution and community structure through different mechanisms (Bolnick et al. 2011), including local adaptation and plasticity (Chevin et al. 2010). However, concerning the simultaneous action of multiple drivers, we still know little about the magnitude of variation in physiological responses, in particular for coastal-marine species (Carter et al. 2013, Applebaum et al. 2014, Spitzner et al. 2019). High levels of variation are likely to characterise species distributed over wide spatial scales in a heterogeneous coastal habitat. Thus, a key question is whether coastal populations experiencing contrasting environmental conditions will be able to persist in a scenario of climate-driven changes.

This is the case of the shore crab *Carcinus maenas*, distributed along the salinity gradient existing between the North and the Baltic Seas (salinity range North Sea: seawater, 30-33 PSU; South Baltic Sea: 10-30 PSU). *C. maenas* is a predator, native to Europe but a global invader elsewhere (Roman & Palumbi 2004). Shore crab larvae from populations of Helgoland in the North Sea (Spitzner et al. 2019) and North Wales in the Irish Sea (Torres et al. 2020), exhibit an antagonistic response to increased temperature and low salinity (defined in Spitzner et al. 2019 as "Thermal Mitigation of Low Salinity stress: TMLS"), by which the negative effects of low salinity on survival and developmental rates are mitigated at higher temperatures. TMLS occurs in other coastal crustaceans where the negative effects of low salinity on survival or physiological performance are mitigated at high temperatures (e.g. Janas & Spicer 2008, Nasrolahi et al. 2012). In the local population from Helgoland, TMLS was found in larvae hatched from embryos kept in seawater, which corresponds to the natural conditions. TMLS may occur through several mechanisms. For instance, capacity to osmoregulate is one such

explanation for a number of other species (Williams 1960, Hagerman & Uglow 1983, Janas & Spicer 2008). Likewise, the larval stages zoea I and megalopa of *C. maenas* from the Helgoland population exhibit increased capacity to osmoregulate when exposed to higher temperatures (Torres et al. 2021). Increased capacity to osmoregulate may reflect a higher rate of pumping ions by the Na^+-K^+-ATPase in the ionocytes, located in the ion-transport tissues, as well as increased production of ATP in the mitochondria (Pörtner 2010). In addition, when performance is quantified as survival to a given stage (e.g. to megalopa), the mitigation effect may occur because, at high temperatures, larvae are exposed to suboptimal salinity for a shorter time (i.e. "phenological effect" in: Torres et al. 2021).

Larvae of *C. maenas* and other marine invertebrates are considered very sensitive to environmental changes (Przeslawski et al. 2015, Pandori & Sorte 2019) and poor larval performance due to environmental stress can lead to recruitment failure and population collapse. Hence, TMLS can enable larvae to exploit coastal habitats of moderately reduced salinity (e.g. above 20 PSU for *C. maenas*) and have a central role in population persistence under a warming scenario. TMLS may drive distribution patterns and community structure (Liu et al. 2020, Torres et al. 2021). However, while TMLS may be a trait of local populations distributed in areas influenced by seawater, we still do not know anything about the responses to temperature and salinity in populations located in habitats dominated by brackish water, such as the Baltic Sea. We know that adults of *C. maenas* from the Baltic Sea exhibit increased capacity to osmoregulate as compared to those from the North Sea (Theede 1969), possibly providing increased tolerance to low salinity. Likewise, one would expect that larvae of *C. maenas* from populations located in the Baltic Sea should exhibit increased tolerance to low salinity for the whole life-cycle. If larvae of such populations are well adapted to low salinity, one should find a shift in the optimal salinity (e.g. higher survival at low salinity, e.g. 20 PSU *vs.* seawater, e.g. 32 PSU) or an increase in the degree of euryhalinity (survival is little affected

by low salinity as compared to seawater). From current knowledge, it is very difficult to predict how temperature may modify the response to low salinity for populations located in habitats such as the Baltic Sea, but we should not find the same antagonistic response in larvae from North and Irish Seas populations.

Another important point is the embryos that are subject to similar salinity conditions to the females during embryonic development. Such conditions can drive "post-zygotic maternal effects" (Wade 1998) and thus modify larval responses to salinity and temperature (Giménez & Anger 2003, González-Ortegón & Giménez 2014). In some estuarine species, moderately low salinities experienced during embryonic development enhance larval performance (Charmantier et al. 2002). While in a population of *C. maenas* from the Irish Sea, low salinity (20 PSU) impaired performance (Torres et al. 2020), this should not be the case for a population located in the Baltic Sea where embryos develop under low salinity conditions. Hence, we expect that in populations of the Baltic Sea, low salinity experienced by embryos should at least not impair larval performance as in the case of populations from the Irish and North Sea.

Here, we compared the responses to temperature and salinity in larvae of a population occurring in the Baltic Sea (Kerteminde, Denmark) with those of a North Sea population (Helgoland, German Bight). We carried out a comprehensive study on the effects of salinity and temperature on several larval traits from hatching to metamorphosis. As a proxy for larval performance, we measured survival, duration of development, dry mass, carbon and nitrogen content, and growth rates. In the same experiment, we also quantified the effect of salinity conditions experienced during embryonic development in order to determine how post-zygotic effects may vary between populations according to the salinities experienced by their embryos. In contrast to the larvae from Helgoland (North Sea), we expect that larvae from Kerteminde (Baltic Sea) will be highly tolerant to low salinity, especially after experiencing low salinity during embryogenesis.

2. Materials and methods

2.1. Model species

Carcinus maenas is a coastal-estuarine inhabitant, native to Europe but considered one of the 100 most successful invaders by IUCN (see also Cohen et al. 1995, Carlton & Cohen 2003, Roman & Palumbi 2004, Hidalgo et al. 2005), with known established populations in Australia, America, and Asia (Leignel et al. 2014). *C. maenas* has a biphasic life cycle consisting of bottom living (benthic) adults, four fully pelagic larval stages (zoea I-IV) that disperse in the water column, followed by a metamorphosis to an additional semi-benthic larval stage (megalopa) that colonises the shore habitats (Spitzner et al. 2018). Low salinities can reduce developmental and growth rates in larval stages (Anger et al. 1998, Spitzner et al. 2019, Torres et al. 2002).

2.2. Study design

Females with early-stage embryos were collected from the shores of two locations: North Sea: Helgoland, Germany (salinity = 32 - 33 PSU; coordinates: 54° 10' 49.176" N, 7° 53' 20.198" E) and Baltic Sea: Kerteminde, Denmark (salinity = 16 - 20 PSU; coordinates: 55° 26' 59.9" N, 10° 39' 40.1" E). We first exposed berried females from both populations to two embryonic salinities, i.e. natural salinities in which each population occurs (Fig. 1a). Once the larvae hatched, we quantified performance (survival, developmental time, dry mass, carbon and nitrogen content, and growth rates) after larvae were exposed to different combinations of temperature and salinity (Fig. 1b). Hence, we were able to evaluate the combined effect of population of origin (P), embryonic salinity (E_S), larval temperature (L_T) and salinity (L_S) on larval performance.

Animals of both populations were kept individually in 5 L aquaria. Berried females with early-stage embryos of each population were randomly assigned to two different embryonic salinities (referred as E_S): 20 PSU and 32.5 PSU, which resembled salinity conditions in the natural habitats of the Baltic Sea (Kerteminde) and North Sea (Helgoland), respectively (Fig. 1a). The experimental salinity 20 PSU was chosen to match the maximum salinity registered in the Danish fjord during the time of collection; and 32.5 PSU as the salinity registered for the North Sea around Helgoland. In addition, preliminary experiments with females from the North Sea population showed that females kept at salinities lower than 20 PSU could not complete embryonic development (unpublished data). Upon hatching, larvae were then assigned randomly to different combinations of temperature and salinity following a factorial experimental design (Fig. 1b) based on twelve combinations of four temperatures (L_T; referred as larval temperature): 15, 18, 21, and 24 °C, and three larval salinities (L_S; referred as larval salinities): 20, 25, and 32.5 PSU. Parameter checks after 24 h showed that salinity changed slightly but always was < 1 PSU (increase at 15 and 18 °C: 0.2 - 0.3 PSU; at 21 and 24 °C ~ 0.5 - 0.7 PSU). Larvae were assigned to the treatments in five replicate groups (Fig. 1b). Each replicate group consisted of 60 ml glass bowls with ten random individuals each (i.e. the replicate units were the individual bowls).

The experiments were repeated with larvae from 18 females (i.e. five different females from each population and each embryonic salinity), except for the females exposed to an embryonic salinity of 20 PSU from Helgoland, where only three females had a successful embryonic development (Fig. 1a). Note that individual females are identified as Fem 1-5 for each combination of population and embryonic condition (e.g. see Fig. S1 or S3). Overall, the experiment started with 10800 larvae (= 10 larvae × 5 replicates × 3 larval salinities × 4 larval temperatures × 18 females).

INSERT FIGURE 1

2.3. **Experimental procedures**

Experiments were conducted during the reproductive period of *C. maenas*. Animals from the Kerteminde population were caught using traps in the subtidal fjord next to the Marine Biological Research Centre (University of Southern Denmark, Kerteminde, Denmark). Until transport to the Marine Biological Station at the Alfred-Wegener-Institute (Helgoland, Germany), berried females were kept for a week in a flow through system of natural water from the fjord (temperature: 10 ± 1 °C, salinity: 16 - 20 ± 1 PSU) and gentle aeration. For transport to Germany, organisms were placed individually in plastic containers (volume: 1 L) half-filled with filtered natural seawater from the fjord and a wet towel. To ensure sufficient oxygen concentration during transport (ca. 24 h), the filtered seawater was strongly aerated before filling the containers. Afterwards, the containers with animals were placed in a Coleman® cooler box to ensure a constant temperature (10 - 12 °C) during transport. Upon arrival to the laboratory on Helgoland, the temperature was gradually increased (1 °C per day) to match the temperature in summer for both populations, thus reaching a common embryonic temperature (15 °C). The animals from the Helgoland population were collected manually from the intertidal rocky shore and directly transported to the Marine Biological Station at the Alfred-Wegener-Institute. For these berried females, transport stress was simulated for 24 h in the laboratory aiming to replicate the transport conditions of the animals from Kerteminde, i.e. animals were individually placed for 24 h in half-filled plastic containers with fully aerated natural seawater (natural salinity: 32.5 ± 0.5 PSU) and temperature (15 ± 0.5 °C) with a wet towel. Water was changed daily and animals were fed (frozen shrimp *Crangon crangon*) twice per week. Salinities were adjusted gradually (salinity decrease/increase rate: 2 PSU day^{-1}) until the desired embryonic salinity (20 or 32.5 PSU) was achieved.

Experiments with larvae (Fig. 1b) were run in temperature-controlled rooms (± 0.5 °C) with a 12-12 h light-dark cycle. Filtered (filter pore size = 0.2 µm) aerated natural seawater was used for all experiments, and lower salinities were prepared by diluting natural seawater with appropriate amounts of tap water. Bowls were filled to 75 % of the volume and salinity (E_S and L_S ± 0.5 PSU) was monitored with a salinometer (WTW Cond 3110 SET1). Water and food (*ad libitum* freshly hatched *Artemia sp*., Great Salt Lake Artemia, Sanders, USA) were changed daily. During the daily water change, survival and development of the larvae were monitored; moults and dead larvae were recorded and discarded.

2.4. **Elemental analysis**

In order to determine elemental composition (C and N content) and growth, three replicates of freshly hatched zoea I (50 larvae each) were randomly chosen at the start of the experiment and three replicates of recently moulted megalopa (2 megalopa each) were randomly selected at the end of each experiment. Larvae were gently rinsed with distilled water for ten seconds, blotted dry, placed into aluminium cartridges and stored at -20 °C for further analyses. Prior to the elemental composition analyses, samples were freeze-dried for 48 h (Christ Alpha 1–4 freeze-drier) and dry mass was determined using a microbalance (Sartorius SC2, nearest 0.0001-mg). Elemental composition was determined as carbon and nitrogen content using an Elemental analyser (vario MICRO cube CHNS analyser, Elementar Analysensysteme).

2.5. **Data analysis**

Survival to each stage was calculated as the cumulative proportion of larvae surviving from hatching to each stage. Prior to the analysis, the survival proportions (p) were re-scaled with the formula $p' = [p (N-1) + 0.5]/N$, where N is the number of larvae per glass (= 10 larvae) at the start of the experiment. This transformation is used to avoid situations of log(0) values. Survival proportions were then transformed to a logarithmic and logistic scale. Developmental

time was determined as the time from hatching to reach each larval stage. Dry mass (DW), carbon (C) and nitrogen (N) content were calculated for freshly hatched zoea I and post-moult megalopa. Instant growth was calculated following the formula: $\log(B_{(M)}/B_{(ZI)})/DD$ where B is the biomass parameter (DW, C or N) and DD is the time from hatching ($_{ZI}$) to reach the megalopa ($_{M}$). Means, standard deviations and standard errors were calculated *via* "plyr" package in R (version 3.6.1) through RStudio.

The experimental design had four fixed and orthogonal factors: (1) population: P; (2) embryonic salinity: E_S; (3) larval temperature: L_T, and (4) larval salinity: L_S. In addition, there was a random factor: female of origin (F) nested in the interaction of embryonic salinity and population. The analyses were carried out through mixed models, applying backward model selection (Zuur et al. 2009) using the package nlme (Pinheiro et al. 2019), and the functions lme (for mixed models) and gls (for fixed models).

For mixed modelling, model selection of random terms was carried out by comparing models after restricted maximum likelihood (REML). Then, the fixed terms were examined after refitting the model with the best random structure with maximum likelihood (ML). In all cases, model selection was based on the corrected Akaike information criteria (AICc) and likelihood ratio tests (LRT); Tukey HSD test was used for post-hoc comparisons. When comparing multiple models, if the simplest model had the lowest AICc, it was selected for further analysis. In cases when $\Delta AICc < 3$ and a more complex model had lower AICc, we applied LRT; if models differed significantly ($p < 0.05$), the lower AICc model was selected; in the opposite situation, the model with the lower number of parameters was chosen.

The full mixed model for survival (Table 1) contained two components: (1) the four factorial fixed components combining population, embryonic salinity, larval salinity, and larval temperature ($\sim P \times E_S \times L_S \times L_T$); (2) the interaction terms reflecting different random components. The full random part of the model was initially specified with the full covariance

matrix, but model fitting led to convergence issues, suggesting overfitting. By following the recommendations given in Bolker et al. (2009), we then re-specified the matrix as diagonal, coded as "random = list (F = pdDiag(~L_S × L_T))". For the selection of the random component, there were also more simple models specified with the full covariance matrix (e.g. as "random = list (F = pdSymm(~L_S))" or "random = list (F = pdSymm(~L_T))" or "random = ~1|F"). For duration of development, the four-way interaction (~P × E_S × L_S × L_T) was kept but the lowest level of the factor larval salinity (L_S = 20 PSU) was excluded from the analyses due to insufficient number of surviving larvae to estimate duration of development.

Separate analyses for developmental duration were carried out for the different stages, depending on the survival in the specific treatments. For instance, zoea II and zoea III analyses was performed for full mixed model (Table S1) with larvae reared at L_S = 25 and 32.5 PSU, while duration of the development until megalopa stage was only possible for E_S = 32.5 PSU with larvae developing at L_S = 32.5 PSU which decreased the level of factors (Table S2). Additionally, three-way models were fitted for the megalopae from E_S = 32.5 PSU to check for significant interactions between larval salinities (25 - 32.5 PSU) in the temperatures 18-24 °C where sufficient megalopae survived.

To evaluate biomass of the megalopa, a mixed model for the dry mass, and C and N content was performed. However, the analysis was restricted to larvae originating from seawater (E_S = 32.5 PSU) due to high mortality early in the development of larvae from broods kept at E_S = 20 PSU. Note that L_S = 20 PSU is also excluded from the analysis due to high mortality of the larvae. Therefore, the analysis was divided into two parts. In the first step, we compared both populations with a three-way fixed interaction (~P × L_S × L_T), excluding L_T = 15 °C due to insufficient survivors to megalopa at L_S = 25 PSU in the Baltic Sea population (Table S3). In the second part of the analysis, we included the lowest larval temperature and analysed effects of combined larval salinity and temperature (~L_S × L_T) only for the North Sea population for

all treatments (Table S4). An additional ANOVA was performed also for the Baltic Sea population excluding the lowest temperature (15 °C) to test the effect of larval salinity. In all steps of the biomass data analysis, female of origin (F) was kept as a random factor (coded in the model as: "random = ~1|F").

For the instantaneous growth rates, the starting model had the same structure as the one used for DW, C and N content (Table S3, S4). For the biomass analyses, always two megalopae were merged in one replicate. Thus, we first calculated averages of DW, C and N content of each replicate. Then, we calculated the average DD (i.e. from freshly hatched zoea I to successfully moulted megalopa) of the two megalopae in each replicate, which we then used to calculate instant growth rates specifically.

In addition, we studied the covariation between survival, development, and body mass of larvae as well as responses at different factor combinations. For duration of development, where we had the largest data set, we compared the output of models fitting bivariate responses (accounting for covariation between development and survival) with univariate models for duration of development. We carried out a bivariate analysis in order to account for potential viability selection or the so called invisible fraction (Hadfield 2008). Because mortality may be trait-selective (e.g. survivors may be those characterised by higher developmental rates), animals used to estimate trait values are not sampled at random, but instead are a sample contingent on the population of trait values of the survivors. Those analyses were carried out using generalised mixed linear models based on Monte Carlo Markov chain for parameter estimation, in R, using the package MCMCglmm (more details on that analysis are provided in the Supplement: Section 1).

3. **Results**

For simplicity, we use the terms “North Sea” *vs*. “Baltic Sea” corresponding to the “Helgoland” *vs*. “Kerteminde” populations, but we clarify that we do not assume that these populations are representative of each region, as we recognise (see discussion) that responses may well vary considerably among populations located within each particular region.

3.1. **Larval survival**

Larval survival varied among populations and was driven by the combination of embryonic salinity, larval salinity and larval temperature (Fig. 2, Table 1). Overall, females that produced larvae with high survival at a given temperature and salinity combination also produced larvae with high survival at other combinations (Tables S5 and S6). For the North Sea population, and when embryos developed in seawater, we found an antagonistic response to larval temperature (L_T) and salinity (L_S) consistent with the thermal mitigation of low salinity stress (TMLS) previously reported (Spitzner et al. 2019). The TMLS was found in the survival to the megalopa (Fig. 2a) in larvae reared at moderately low salinity (L_S = 25 PSU): survival dropped down significantly to less than 20 % at low temperatures (15 and 18 °C), but remained high at higher temperatures (> 60 % at 21 and 24 °C) as for larvae reared in seawater. At the lowest larval salinity (L_S = 20 PSU) survival to megalopa was consistently low (Fig. 2a). For this population, the TMLS was initially established at the zoea II stage, in larvae from all females reared at the lowest salinity (Fig. S1). High survival rates were found at moderately low salinity and in seawater, although responses varied among larvae from different females (Fig. S1). The TMLS was strong for survival to the zoea II: average survival was high at the lowest salinity and highest temperature (= 70 %); it was more than two times higher than the expectations of the joint probability (0.7 > 0.28 = 0.4 × 0.7) calculated as the product of the proportion survival at the lowest salinity (= 0.4 at optimal L_T = 15 °C) multiplied by that occurring under 21 or 24 °C (= 0.7 at optimal L_S = 32.5 PSU).

There were four main outcomes in the comparisons among embryonic salinity and population. First, for the North Sea population, low embryonic salinity resulted in an important and significant ($p < 0.001$) reduction in overall survival, a disruption of the TMLS, and a reduction of tolerance to high temperature (Figs. 2b, S2b). The best combination of temperature and salinity (15 °C and seawater) led to only moderate survival (zoea II: 50 %; megalopa: 35-40 %) which was significantly lower than that of larvae hatching from embryos kept in seawater ($p < 0.01$; zoea II: > 80 %; megalopa: 70 %). Larvae reared at low salinities had consistently low survival (Figs. 2b, S2b: no mitigation effect); in addition, increased temperature resulted in reductions in survival for larvae reared in seawater.

Second, larval survival from the Baltic Sea population was lower than that from the North Sea. Larvae reared at the lowest salinity had consistently the lowest survival to Zoea II (Fig. S2c) irrespective of the female of origin (Figs. S4, S5).There was also a weak TMLS, in larvae originated from embryos reared in seawater and exposed to moderately low salinity (Figs. 2c, S2c); this weak response reflected a high variability among females in the effect of temperature on survival (Fig. S3).

Third, there were differences among populations in how embryonic salinity affected larval survival. In larvae from the Baltic Sea population, hatching from embryos exposed to low salinity, survival was consistently low irrespective of temperature, unlike the pattern observed for larvae from the North Sea (Figs. 2, S2).

Fourth, for the Baltic Sea population, larval survival did not peak at the lowest salinity, expected to be experienced in the field. In addition, when embryos were kept in seawater, the highest survival occurred at the higher salinities (25 and 32.5 PSU compared to 20 PSU; for zoea II: $p < 0.001$). When embryos were kept at low salinity, survival was low irrespective of the salinity experienced by larvae (Figs. 2d, S2d). Lowest survival rates occurred at low

embryonic (E_S = 20 PSU) and larval salinity (L_S = 20 PSU), which are the salinities experienced in the natural habitat.

INSERT TABLE 1

FIGURE 2

3.2. **Developmental time**

Developmental time decreased at higher temperatures and increased at lower salinities, but also varied among population of origin (Figs. 3, 4). There were three main responses in the developmental time to the zoea II. First, there was a clear effect of high temperature in decreasing developmental time, which varied slightly among salinities and populations (Fig. 3). Second, the effect of larval salinity (a 1-3 days increase in developmental time) was only present in larvae from the North Sea hatched from embryos kept at low salinity (Fig. 3b). Third, for both populations, low embryonic salinity resulted in longer larval development especially at low temperature (5-7 longer at 15 °C, $p < 0.001$ *vs*. ca. 3.5 days longer at 24 °C, $p < 0.05$).

For the megalopa, the best models retained interactions between the three terms tested ($L_T \times L_S$ and $P \times L_S$; embryonic salinity, E_S = 20 PSU was not considered due to low survival). Developmental time to the megalopa showed a pattern consistent with TMLS (Fig. 4) especially for larvae from the North Sea population. In that population, at 25 PSU, developmental time was 5 days longer at 15 °C ($p < 0.001$), but there was no significant delay in development at 21-24 °C compared to larvae reared at 32.5 PSU. In addition, larvae from the North Sea had in general lower developmental times than those of the Baltic Sea population.

Bivariate models (based on MCMCglmm) indicated that covariances between developmental time and survival included zero in the credible interval for the random structure

(Table S7), but not for the error structure (Table S8). Correlations between survival and developmental time were mostly negative, indicating that faster development was associated with higher survival (Fig. S6). In general, comparisons of results between bivariate and univariate models led to similar credible intervals (Tables S9-S11) except of a single estimate (Table S9). Overall, our interpretation is that the conclusions drawn from univariate analyses were robust to covariation between survival and developmental time.

INSERT FIGURES 3 & 4

3.3. **Biomass and elemental composition**

Dry mass (DW), carbon (C) and nitrogen (N) content of the freshly hatched zoea I varied between populations and embryonic salinity treatments (Fig. 5). When embryos were kept in seawater, newly hatched larvae from the North Sea population had significantly higher DW, C and N content as compared to those from the Baltic Sea population (e.g. DW in the North Sea *vs.* Baltic Sea population: 10.7 *vs.* 8.12 µg ind^{-1} respectively, i.e. ~30 % lower in the Baltic Sea population). In the North Sea population, low embryonic salinity resulted in a reduction by ~20-30 % of DW, C and N content ($p < 0.05$). For the Baltic Sea, embryonic salinity conditions did not affect body mass or reserves at hatching.

INSERT FIGURE 5

The dry mass and elemental composition of the megalopa varied between populations and responded to the larval conditions (Figs. 6, S7); note that all megalopa developed from larvae originated from embryos kept in seawater. The best statistical model retained the three-way factorial interaction $P \times L_S \times L_T$ for the interpopulation comparison (Table S3), and two-way interaction $L_S \times L_T$ for analysis of the North Sea population (Table S4). For the North Sea population, the combined effect of larval temperature and salinity was consistent with a TMLS: low salinity resulted in a decrease in biomass at low temperatures (24-26 %, Fig. 6), but such

reduction was smaller at higher temperatures (<15 %), especially in terms of carbon and nitrogen content (Fig. S7). By contrast, in larvae from the Baltic Sea population, low salinity had a consistent negative effect on dry mass and elemental composition, irrespective of temperature (Figs. 6, S7). In addition, dry mass, C and N content were higher in larvae from the North Sea population than those from the Baltic Sea, but such differences were significant only for C content (see Fig. S7, top panels) in larvae reared at low salinity and at 24 °C (overall, larvae from the North Sea population had 15.9 % higher carbon content). The C:N ratios (Fig. S8) did not indicate any proportional change on the C or N fractions regardless of the population of origin or in response to any treatments. Correlations between survival and dry mass were either positive or non-significant, indicating that high larval survival was associated with large body mass (Fig. S9).

Instantaneous growth rates from hatching to megalopa increased with temperature in both populations (Figs. 6, S7). For the North Sea population, growth rates showed a pattern consistent with TMLS, as in the case of body mass and duration of development, i.e. reductions in growth rates were found only for the combination of low temperature and salinity. Larvae from the Baltic Sea population were less affected by low salinity with a significant reduction only at 18 °C, for the dry mass ($p < 0.05$, Fig. 6). Significant differences between populations appeared only at low salinity and at 18 °C where larvae from the North Sea population had ~25 % lower instantaneous growth rates than those from the Baltic Sea (Figs. 6, S7).

INSERT FIGURE 6

The integrated responses of growth, developmental time and body size are shown in Figures 7 and S10. For the North Sea, the TMLS is now observed as an integrated response, especially in the carbon and nitrogen content. Larvae reached an upper body mass threshold when reared in seawater, especially at the highest temperatures (e.g. at 21 and 24 °C: C ~34-36 μg ind^{-1}, DW ~90 μg ind^{-1}); almost the same thresholds were reached when reared at low

salinity and the highest tested temperatures without any increase in developmental time. By contrast, at lower salinities and temperatures, larvae metamorphosed at lower biomass thresholds in spite of extended development. For the Baltic Sea population, patterns were not consistent with TMLS; the maximum body mass (e.g. at 21 and 24 °C: C ~32-34 µg ind^{-1}, DW ~85 µg ind^{-1}) was reached by larvae reared in seawater; those reared at low salinity were not able to reach that threshold irrespective of an extension in developmental time.

INSERT FIGURE 7

4. Discussion

Here, we show that responses to temperature and salinity can vary considerably between populations of the same species. Using females, collected during the same reproductive season, we confirmed that most larvae hatching from females collected on Helgoland (North Sea) exhibited thermal mitigation of low salinity stress (TMLS), which is consistent with a previous study by Spitzner et al. (2019) for the same population (females collected in consecutive previous years, 2016 and 2017). In addition, the higher survival in seawater is consistent with the fact that the larvae from the population of Helgoland are likely to develop under seawater conditions. For the Kerteminde population (Baltic Sea), we hypothesised that larvae would show a shift in the pattern of tolerance towards low salinities or an increase in the degree of euryhalinity. However, the best survival occurred in seawater instead of at the lowest salinities tested, especially in larvae originated from females kept at low salinity; survival decreased towards lower salinity and higher temperature. The response to low salinity was surprising because embryos as well as larvae of Kerteminde should experience similar salinities as the adults (i.e. ~15-20 PSU).

For the Helgoland population, TMLS was exhibited in terms of the integrated effects on developmental time, body mass and growth rate. At high temperatures, instantaneous growth and developmental rates were not affected by salinity. From the ecological standpoint, the observed traits values of the survivors after developing at low salinities (reduced body mass at metamorphosis), may reduce post-metamorphic survival (Pechenik et al. 1998, Pechenik 2006, Torres et al. 2016); such effects would be minimised under increased temperatures, as long as larvae are not food-limited (Torres & Giménez 2020). Overall, for the Helgoland population, we report for the first time, a consistent mitigation effect of high temperatures on physiology and survival that may favour the use of estuarine habitats, at least for limited time periods, under warming scenarios. By contrast, for the Kerteminde population, negative effects of low salinity on instantaneous growth dominated the response of body reserves at metamorphosis. Hence, in addition to the reductions of larval survival, one would expect ecological consequences of low salinity after metamorphosis (associated to reduced body mass) consisting in reduced post-metamorphic survival. The general low tolerance to low salinity of larvae from Kerteminde is striking and may be considered maladaptive, given that the Baltic Sea is characterised by low salinity.

Because both populations are located at sites differing in the surrounding salinities, we hypothesised that the salinity experienced during embryogenesis could modify the larval responses to temperature and salinity in a way that is adaptive to each population. We knew that such type of post-zygotic maternal effect could have profound effects in salinity tolerance (Giménez & Anger 2003) by increasing osmoregulatory capacity (Charmantier et al 2002), as osmoregulation provides buffering effects of low salinity on larval performance (Torres et al. 2011). Hence, for the Kerteminde population, we expected that low salinity experienced by embryos would enhance larval performance at low salinities, or at least not impair it. However, low embryonic salinity caused reduction of larval performance in the Kerteminde population,

contrary to our expectations. Hence, the post-zygotic maternal effects of the local population of Kerteminde may be considered maladaptive.

We know that adults of *C. maenas* exhibit adaptive responses to low salinity conditions in the Baltic Sea (Theede 1969). In addition, larvae of populations from the coastal waters of the North Sea *vs.* the Baltic Sea exhibit different behavioural traits, that are adaptive for the different hydrodynamic conditions experienced in each sea (Moksness et al. 2014: comparison of larval vertical migration patterns). Then, why does larval tolerance not exhibit patterns that are adaptive to the salinity conditions surrounding the Kerteminde population? We do not know if the maladaptive response of the Kerteminde population is characteristic of other Baltic Sea populations or driven by local conditions characterising the Kerteminde Fjord (e.g. maternal nutrition, presence of additional drivers). Any other known responses for the European continent, experiencing comparable salinity conditions, (Isle of Man, UK: Nagaraj 1993; North Wales, UK: Torres et al. 2020; Cádiz, Spain: unpublished data), are similar to the one found for the Helgoland population. Maladaptive responses to temperature and salinity have however been found in populations of an estuarine barnacle, also occupying habitats in the Baltic Sea (Nasrolahi et al. 2016). Maladaptive responses may be maintained by gene flow (Kawecki & Stearns 1993, Bolnick & Nosil 2007, Farkas et al. 2016), sustained by larvae arriving to the Kerteminde Fjord from other local populations of the Baltic Sea or perhaps from the North Sea. Perhaps, Kerteminde harbours a sink population; in theory, subsidy from sink to source populations can contribute to species distribution in areas characterised by environmental gradients (Dauphinais et al. 2018, Giménez et al. 2020).

Alternatively, larvae sustaining the population from Kerteminde develop in microhabitats characterised by increased salinity or they develop under temperature conditions that are much lower than those tested here. Larvae may develop in deeper waters characterised by higher salinities and perhaps lower temperatures. While females in the North Sea are found in the

intertidal area, those of the Baltic Sea are subtidal; perhaps larvae stay close to the bottom. However, studies of larval behaviour suggest diel vertical migrations (from bottom to surface waters) in larvae from Baltic Sea populations (Moksnes et al. 2014), which would predict that larvae should occupy near-surface waters with reduced salinity, at least for limited periods. In addition, the overall differences in larval performance would suggest increased larval mortality in those produced by the population in Kerteminde, irrespective of temperature and salinity. Overall, given the current evidence, it is difficult to envisage a scenario other than the one in which the local population of Kerteminde is subsidised from other nearby populations (taken into consideration that larvae are the main dispersal stages in this species).

A potential physiological driver for the reduced performance in the Kerteminde population could be the reduced body mass at hatching, which coincided with that observed in larvae from the Helgoland population hatching from embryos kept at low salinity. Poor performance associated to low body mass at hatching has been found in previous studies (Giménez & Anger 2001, Marshall & Keough 2007, González-Ortegón & Giménez 2014, Oliphant et al. 2014). Reduced larval body mass, a proxy for body size, is associated with lower metabolic efficiency (Pettersen et al. 2015, Marshall et al. 2018) and it is likely to constrain the capacity to capture prey. Perhaps drivers of reduced body mass (e.g. local food availability at the time of maternal allocation of reserves to eggs) are responsible for the overall poor larval performance of the Kerteminde population.

In synthesis, irrespective of the underpinning mechanisms, our study highlights important differences among populations of the same species in the capacity to cope with various salinity and temperature combinations. Hence, when asking questions about “winners” or “losers” (Somero 2010) we cannot make judgements based on single population studies. For the Helgoland population, increased larval performance is found consistently under salinity conditions of the North Sea. However, the responses to low salinity found for the Kerteminde

population are maladaptive for the natural conditions of the Baltic Sea and represent a form of "ecological surprise" (Filbee-Dexter et al. 2017), highlighting the fact that adaptive responses should not be expected by default. We emphasise the importance of incorporating a multi-population approach and considering effects of the maternal environment on offspring responses in multiple stressors research. By expanding the spatial scale of observation, from local to regional, we could obtain a more complete picture of species responses to climate-driven changes in environmental variables.

Author Contributions: *Conceptual development*: LG, SH and GT; *study design*: LG, ZS and GT; *resources*: SH, JK, KF and GT; *data acquisition*: ZS and GT; *data analysis and interpretation*: LG, ZS and GT; *first draft writing*: ZS, *review and editing of submitted manuscript*: all authors.

Acknowledgements: We wish to thank Dr. Magnus Wahlberg (University of Southern Denmark, Denmark) for access to the Kerteminde population. We gratefully acknowledge Julia Haafke (AWI) for performing the elemental analysis and Siri Rohwer (University of Greifswald) for help with larval rearing. This study is part of ZS doctoral dissertation and was supported by the Deutsche Forschungsgemeinschaft (Research Training Group 2010: RESPONSE) at the University of Greifswald, in a collaboration with the Alfred-Wegener-Institute.

Conflict of interest: The authors declare no competing interests. The funding body did not influence the design of the study, collection, analysis and interpretation of data, nor the writing of the manuscript.

Permits: The research presented in this paper complies with the guidelines from the directives 2010/63/ EU of the European parliament and of the Council of 22nd September 2010 on the protection of animals used for scientific purposes.

References

Andersson A, Meier HEM, Ripszam M, Rowe O, Wikner J, Haglund P, Eilola K, Legrand C, Figueroa D, Paczkowska J, Lindehoff E, Tysklind M, Elmgren R (2015) Projected future climate change and Baltic Sea ecosystem management. AMBIO 44:345-356.

Anger K, Spivak E, Luppi T (1998) Effects of reduced salinities on development and bioenergetics of early larval shore crab, *Carcinus maenas*. J Exp Mar Biol Ecol 220:287-304.

Applebaum SL, Pan T-CF, Hedgecock D, Manahan DT (2014) Separating the nature and nurture of the allocation of energy in response to global change. Integr Comp Biol 54(2):284-295.

BACC I (2008) Assessment of climate change for the Baltic sea basin. Springer, Berlin.

Boersma M, Grüner N, Signorelli NT, González PEM, Peck MA, Wiltshire KH (2016) Projecting effects of climate change on marine systems: is the mean all that matters? Proc R Soc B 283:20152274.

Bolnick DI, Nosil P (2007) Natural selection in populations subject to a migration load. Evol 61:2229-2243.

Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vassuer D (2011) Why intraspecific trait variation matters in community ecology. Trends Ecol Evol 26(4):183-192.

Boyd PW, Collins S, Dupont S, Fabricius K, Gattuso J-P, Havenhand J, Hutchins DA, Riebesell U, Rintoul MS, Vichi M, Biswas H, Ciotti A, Gao K, Gehlen M, Hurd CL, Kurihara H, McGraw CM, Navarro JM, Nilsson GE, Passow U, Pörtner H-O (2018) Experimental

strategies to assess the biological ramifications of multiple drivers of global ocean change - a review. Glob Chang Biol 24: 2239-2261.

Breitburg DL, Baxter JW, Hatfield CA, Howarth RW, Jones CG, Lovett GM, Vigand C (1998) Understanding effects of multiple stressors: ideas and challenges. In: Pace ML, Groffman PM (eds) Successes, limitations, and frontiers in ecosystem science. Springer, New York, p 416-431.

Browne RA, Wanigasekera G (2000) Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia.* J Exp Mar Biol Ecol 244:29-44.

Burrows MT, Schoeman DS, Buckley LB, Moore P, Poloczanska ES, Brander KM, Brown C, Bruno JF, Duarte CM, Halpern BS, Holding J, Kappel CV, Kiessling W, O'Connor MI, Pandolfi JM, Parmesan C, Schwing FB, Sydeman WJ, Richardson AJ (2011) The pace of shifting climate in marine and terrestrial ecosystems. Science 334:652-655.

Carlton JT, Cohen AN (2003) Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. J Biogeogr 30:1809-1820.

Carter HA, Ceballos-Osuna L, Miller NA, Stillman JH (2013) Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab *Petrolisthes cinctipes*. J Exp Biol 216:1412-1422.

Charmantier G, Giménez L, Charmantier-Daures M, Anger K (2002) Ontogeny of osmoregulation, physiological plasticity and larval export strategy in the grapsid crab *Chasmagnathus granulata* (Crustacea, Decapoda). Mar Ecol Prog Ser 229:185-194.

Chevin L-M, Lande R, Mace GM (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biol 8(4):e1000357.

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecol Lett 11:1304-1315.

Cohen AN, Carlton JT, Fountain MC (1995) Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. Mar Biol 122:225-237.

Dauphinais JD, Miller LM, Swanson RG, Sorensen PW (2018) Source–sink dynamics explain the distribution and persistence of an invasive population of common carp across a model Midwestern watershed. Biol Invasions 20:1961-1976.

Farkas TE, Mononen T, Comeault AA, Nosil P (2016) Observational evidence that maladaptive gene flow reduces patch occupancy in a wild insect metapopulation. Evol 70(12):2879-2888.

Filbee-Dexter K, Pittman J, Haig HA, Alexander SM, Symons CC, Burke MJ (2017) Ecological surprise: concept, synthesis, and social dimensions. Ecosphere 8:e02005.

Folt CL, Chen CY, Moore MV, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnol Oceanogr 44(3, part 2):864-877.

Giménez L, Anger K (2001) Relationships among salinity, egg size, embryonic development, and larval biomass in the estuarine crab *Chasmagnathus granulata* Dana, 1851. J Exp Mar Biol Ecol 260:241-257.

Giménez L, Anger K (2003) Larval performance in an estuarine crab *Chasmagnathus granulata*, is a consequence of both larval and embryonic experience. Mar Ecol Prog Ser 249:251-264.

Giménez L, Exton M, Spitzner F, Meth R, Ecker U, Jungblut S, Harzsch S, Saborowski R, Torres G (2020) Exploring larval phenology as predictor for range expansion in an invasive species. Ecography 43:1423-1434.

González-Ortegón E, Giménez L (2014) Environmentally mediated phenotypic links and performance in larvae of a marine invertebrate. Mar Ecol Prog Ser 502:185-195.

Hadfield JD (2008) Estimating evolutionary parameters when viability selection is operating. Proc R Soc B 275(1635): 723-734

Hänninen J, Vuorinen I, Hjelt P (2000) Climatic factors in the Atlantic control the oceanographic and ecological changes in the Baltic Sea. Limnol Oceanog 45(3):703-710.

Hidalgo FJ et al. (2005) A prediction come true: the green crab invades the Patagonian coast. Biol Invasions 7:547-552.

Hiddink JG, Barón PJ, Orensanz JM (2015) Temperature tracking by North Sea benthic invertebrates in response to climate change. Glob Chang Biol 21:117-129.

Holt J, Schrum C, Cannaby H, Daewel U, Allen I, Artioli Y, Bopp L, Butenschon M, Fach BA, Harle J, Pushpadas D, Salihoglu B, Wakelin S (2016) Potential impacts of climate change on the primary production of regional seas: a comparative analysis of five European seas. Prog Oceanogr 140:91-115.

Janas U, Spicer JI (2008) Does the effect of low temperature on osmoregulation by the prawn *Palaemon elegans* Rathke, 1837 explain winter migration offshore? Mar Biol 153:937-943.

Kawecki TJ, Stearns SC (1993) The evolution of life histories in spatially heterogeneous environments: optimal reaction norms revisited. Evol Ecol 7:155-174.

Ko GWK, Dineshram R, Campanati C, Chan VBS, Havenhand J, Thiyagarajan V (2014) Interactive effects of ocean acidification, elevated temperature, and reduced salinity on early-life stages of the pacific oyster. Environ Sci Technol 48(17):10079-10088.

Le Pape O, Bonhommeau S (2015) The food limitation hypothesis for juvenile marine fish. Fish 16:373-398.

Leignel V, Stillman JH, Baringou S, Thabet R, Metais I (2014) Overview on the European green crab *Carcinus* spp. (Portunidae, Decapoda), one of the most famous marine invaders and ecotoxicological models. Environ Sci Pollut Res 21:9129-9144.

Liu T-X, Kinn-Gurzo S, Chan KYK (2020) Resilience of invasive tubeworm (*Hydroides dirampha*) to warming and salinity stress and its implications for biofouling community dynamics. Mar Biol 167:145.

Marshall DJ, Keough MJ (2007) The evolutionary ecology of offspring size in marine invertebrates. Adv Mar Biol 53:1-60.

Marshall DJ, Pettersen AK, Cameron H (2018) A global synthesis of offspring size variation, its eco-evolutionary causes and consequences. Funct Ecol 32:1436-1446.

Meier HEM, Müller-Karulis B, Andersson HC, Dieterich C, Eilola K, Gustafsson BG, Höglund A, Hordoir R, Kuznetsov I, Neumann T, Ranjbar Z, Savchuk OP, Schimanke S (2012) Impact of climate change on ecological quality indicators and biogeochemical fluxes in the Baltic Sea: a multi-model ensemble study. AMBIO 41:558-573.

Moksnes P-O, Corell H, Tryman K, Hordoir R, Jonsson PR (2014) Larval behavior and dispersal mechanisms in shore crab larvae (*Carcinus maenas*): local adaptations to different tidal environments? Limnol Oceanogr 59(2):588-602.

Moran EV, Hartig F, Bell DM (2016) Intraspecific trait variation across scales: implications for understanding global change responses. Glob Chang Biol 22:137-150.

Nagaraj M (1993) Combined effects of temperature and salinity on the zoeal development of the green crab, *Carcinus maenas* (Linnaeus, 1758) (Decapoda: Portunidae). Sci Mar 57(1):1-8.

Nasrolahi A, Pansch C, Lenz M, Wahl M (2012) Being young in a changing world: how temperature and salinity changes interactively modify the performance of larval stages of the barnacle *Amphibalanus improvisus*. Mar Biol 159(2):331-340.

Nasrolahi A, Havenhand J, Wrange A-L, Pansch C (2016) Population and life-stage specific sensitivities to temperature and salinity stress in barnacles. Sci Rep 6:32263.

Neumann T (2010) Climate change effects on the Baltic Sea ecosystem: a model study. J Mar Syst 81(3):213-224.

Oliphant A, Ichino MC, Thatje S (2014) The influence of per offspring investment (POI) and starvation on larval developmental plasticity within the palaemonid shrimp, *Palaemonetes varians*. Mar Biol 161:2069-2077.

Orr JA, Vinebrooke RD, Jackson MC, Kroeker KJ, Kordas RL, Mantyka-Pringle C, Van der Brink PJ, De Laedner F, Stoks R, Holmstrup M, Matthaei CD, Monk WA, Penk MR, Leuzinger S, Schäfer RB, Piggot JJ (2020) Towards a unified study of multiple stressors: divisions and common goals across research disciplines. Proc R Soc B 287:20200421.

Pandori LLM, Sorte CJB (2019) The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. Oikos 128:621-629.

Pechenik JA, Wendt DE, Jarrett JN (1998) Metamorphosis is not a new beginning: larval experience influences juvenile performance. BioScience 48(11):901-910.

Pechenik JA (2006) Larval experience and latent effects - metamorphosis is not a new beginning. Integr Comp Biol 46(3):32-333.

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. Science 308(5730):1912-1915.

Pettersen AK, White CR, Marshall DJ (2015) Why does offspring size affect performance? Integrating metabolic scaling with life-history theory. Proc R Soc B 282:20151946.

Piggott JJ, Townsend CR, Matthaei CD (2015) Reconceptualizing synergism and antagonism among multiple stressors. Ecol Evol 5(7):1538-1547.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2019) nlme: Linear and Nonlinear Mixed Effects Models. R-package version 3.1-140, http://CRAN.R-project.org/package=nlme.

Poloczanska ES, Brown CJ, Sydeman WJ, Kiessling W, Schoeman DS, Moore PJ, Brander K, Bruno JF, Buckley LB, Burrows MT, Duarte CM, Halpern BS, Holding J, Kappel CV, O'Connor MI, Pandolfi JM, Parmesan C, Schwing F, Thompson SA, Richardson AJ (2013) Global imprint of climate change on marine life. Nat Clim Change 3:919-925.

Pörtner H-O (2010) Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J Exp Biol 213: 881-893.

Przesławski R, Byrne M, Mellin C (2015) A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. Glob Chang Biol 21(6):2122-2140

Reusch TBH (2013) Climate change in the oceans: evolutionary versus phenotypically plastic responses of marine animals and plants. Evol Appl 7(1):104-122.

Robins PE, Skov MW, Lewis MJ, Giménez L, Davies AG, Malham SK, Neill SP, McDonald JE, Whitton TA, Jackson SE, Jago CF (2016) Impact of climate change on UK estuaries: a review of past trends and potential projections. Estuar Coast Shelf Sci 169:119-135.

Roman J, Palumbi SR (2004) A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. Mol Ecol 13:2891-2898.

Sinclair BJ, Ferguson LV, Salehipour-shirazi G, MacMillan HA (2013) Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. Integr Comp Biol 53(4):545-556.

Somero GN (2005) Linking biogeography to physiology: evolutionary and acclimatory adjustments of thermal limits. Front Zool 2:1.

Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J Exp Bio 213(6):912-920.

Spitzner F, Meth R, Krüger C, Nischik E, Eiler S, Sombke A, Torres G, Harzsch S (2018) An atlas of larval organogenesis in the European shore crab *Carcinus maenas* L. (Decapoda, Brachyura, Portunidae). Front Zool 15:27.

Spitzner F, Giménez L, Meth R, Harzsch S, Torres G (2019) Unmasking intraspecific variation in offspring responses to multiple environmental drivers. Mar Biol 166:112.

Stillman JH, Somero GN (1996) Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution. J Exp Biol 199(8):1845-1855.

Stillman JH, Somero GN (2000) A comparative analysis of the upper thermal tolerance limits of Eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. Physiol Biochem Zool 73(2):200-208.

Sulkin S, Blanco A, Chan J, Bryant M (1998) Effects of limiting access to prey on development of first zoeal stage of the brachyuran crabs *Cancer magister* and *Hemigrapsus oregonensis*. Mar Biol 131:515-521.

Tekin E, Diamant ES, Cruz-Loya M, Enriquez V, Singh N, Savge VM, Yeh PJ (2020) Using a newly introduced framework to measure ecological stressor interactions. Ecol Lett 23:1391-1403.

Telesh I, Schubert H, Skarlato S (2013) Life in the salinity gradient: discovering mechanisms behind a new biodiversity pattern. Estuar.Coast Shelf Sci 135:317-327.

Theede H (1969) Einige neue Aspekte bei der Osmoregulation von *Carcinus maenas*. Mar Biol 2:114-120.

Tinker J, Lowe J, Pardaens A, Holt J, Barciela R (2016) Uncertainty in climate projections for the 21st century northwest European shelf seas. Prog Oceanogr 148:56-73.

Todgham AE, Stillman JH (2013) Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. Integr Comp Biol 53(4):539-544.

Torres G, Giménez L (2020) Temperature modulates compensatory responses to food limitation at metamorphosis in a marine invertebrate. Funct Ecol 34:1564–1576.

Torres G, Giménez L, Anger K (2002) Effects of reduced salinity on the biochemical composition (lipid, protein) of zoea 1 decapod crustacean larvae. J Exp Mar Biol Ecol 277:43-60.

Torres G, Giménez L, Anger K (2011) Growth, tolerance to low salinity, and osmoregulation in decapod crustacean larvae. Aquat Biol 12:249-260.

Torres G, Giménez L, Pettersen AK, Bue M, Burrows MT, Jenkins SR (2016) Persistent and context-dependent effects of the larval feeding environment on post-metamorphic performance through the adult stage. Mar Ecol Prog Ser 545:147–160.

Torres G, Thomas DN, Whiteley NM, Wilcockson D, Giménez L (2020) Maternal and cohort effects modulate offspring responses to multiple stressors. Proc R Soc B 287:20200492.

Torres G, Charmantier G, Wilcockson D, Harzsch S, Giménez L (2021). Physiological basis of interactive responses to temperature and salinity in coastal marine invertebrate: Implications for responses to warming. Ecol Evol 11, 7042-7056.

Violle C, Reich PB, Pacala SW, Enquist BJ, Kattge J (2014) The emergence and promise of functional biogeography. Proc Natl Acad Sci 111(38):13690–13696.

Wade M (1998) The evolutionary genetics of maternal effects. In: Mousseau TA, Fox CW (eds) Maternal effects as adaptations. Oxford Univ. Press, New York, p 5-21.

Wiltshire KH, Kraberg A, Bartsch I, Boersma M, Franke H-D, Freund J, Gebühr C, Gerdts G, Stockmann K, Wichels A (2010) Helgoland Roads, North Sea: 45 years of change. Estuaries Coast 33:295–310.

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer-Verlag, New York.

Table 1. *Carcinus maenas*. Larval survival (logarithmic and logistic transformed data) in response to population (P), embryonic salinity (ES), larval temperature (LT), larval salinity (LS) and female of origin (F). F is a random factor representing maternal effects, nested in the interaction P × ES. The remaining four factors are fixed and form a 4-way factorial design. Model selection on random terms was carried out through restricted maximum likelihood (REML) fitting; in all cases the full model performed considerably better than any alternative model. Fixed effects were tested after maximum likelihood (ML) fitting and the full fixed model was retained (P ×ES ×LS ×LT). The best model, therefore, contained both the random and fixed parts (marked in bold).

Model selection:	**Logarithmic**				**Logistic**			
	ZII	**ZIII**	**ZIV**	**M**	**ZII**	**ZIII**	**ZIV**	**M**
Random (REML)								
$F \times L_T \times L_S$	1772	1704	1570	1685	2691	2633	2500	2462
$F \times L_S$	1841	1844	1740	1865	2771	2765	2666	2650
$F \times L_T$	2000	1945	1845	1952	2920	2864	2779	2785
F	2023	1980	1887	2000	2940	2886	2809	2819
No random term	2328	2323	2233	2237	3398	3381	3256	3095
Fixed (ML)								
4-way (full model)	**1695**	**1631**	**1490**	**1604**	**2659**	**2602**	**2463**	**2415**
3-way factorial	1723	1663	1549	1631	2682	2629	2501	2434

Figures

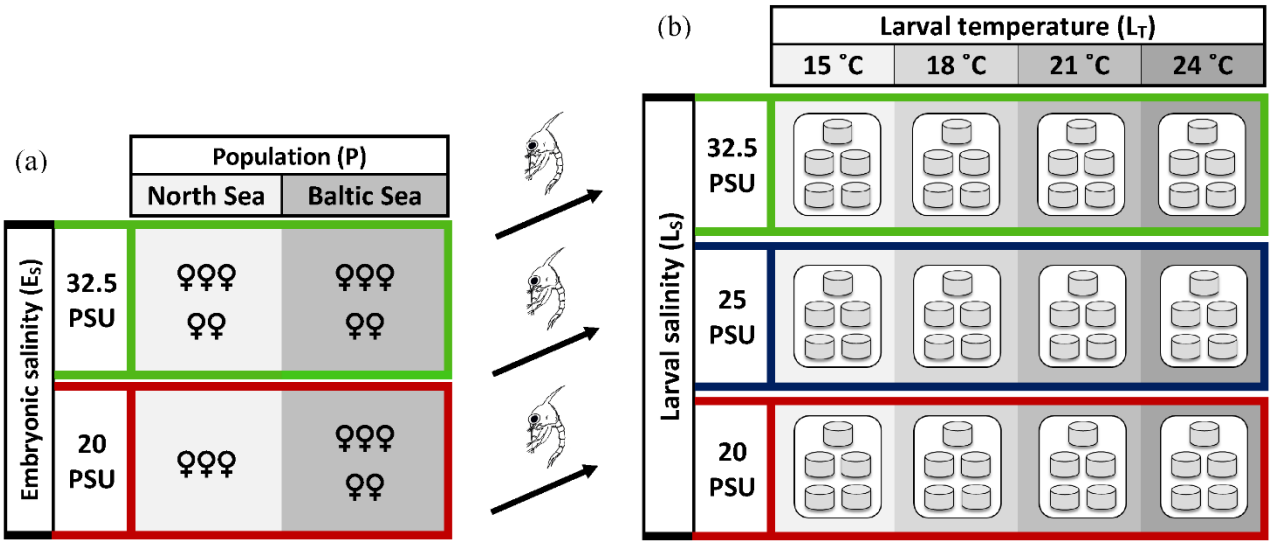

Figure 1. *Carcinus maenas*. Experimental design to test larval performance. (a) Maternal conditions: females (♀) with early-stage embryos from both populations (P) were exposed to different embryonic salinities (E_S) corresponding to the natural habitat salinity of each population (Baltic Sea: 20 PSU, North Sea: 32.5 PSU). Number of symbols (♀) corresponds to the number of females with successfully produced larvae for each combination of P × E_S. (b) Larval conditions: freshly hatched larvae from each female were tested in a factorial experimental design consisted of twelve combinations of larval temperature, L_T (15, 18, 21 and 24 °C) and salinity, L_S (20, 25 and 32.5 PSU) in a common garden setup, to determine larval survival, development, biomass and growth. Each treatment had five replicate 60 ml bowls with ten randomly assigned larvae in each.

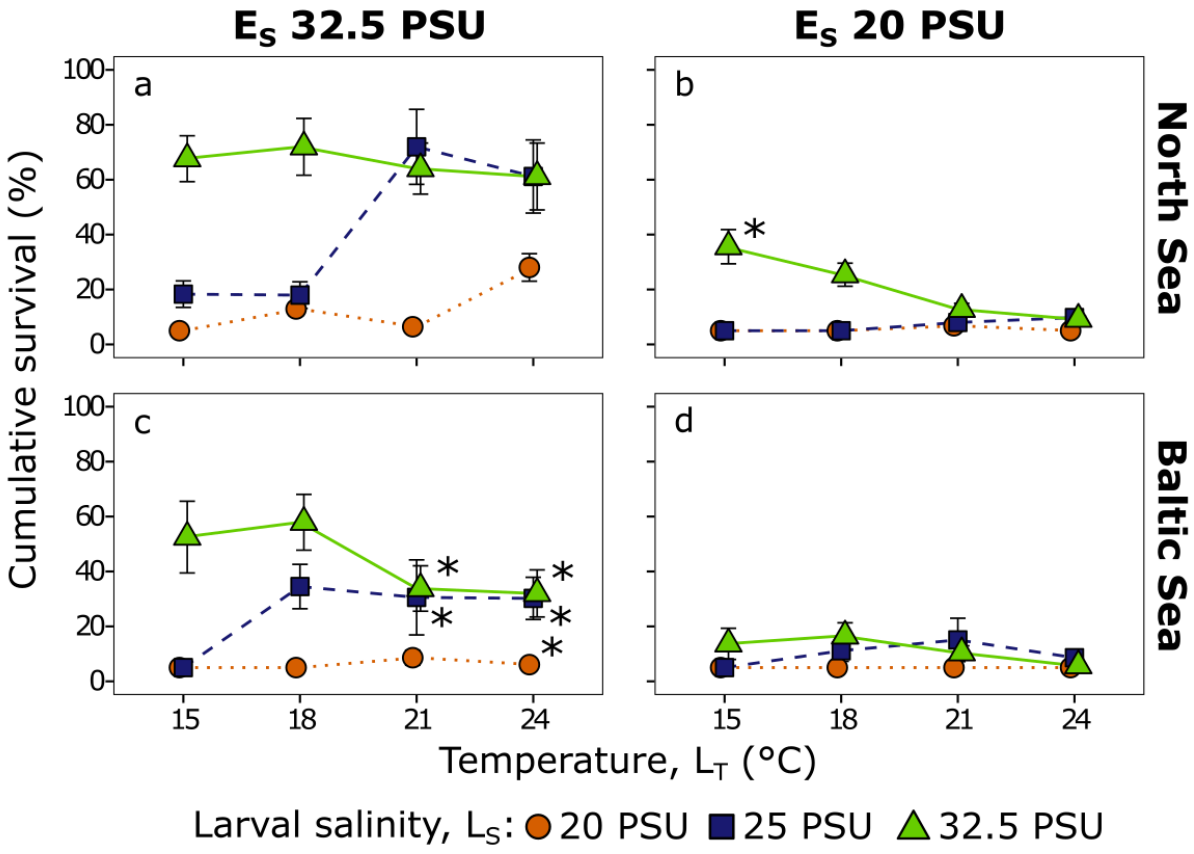

Figure 2. *Carcinus maenas*. Cumulative average survival to megalopa. Comparison between populations (North Sea: a, b; Baltic Sea: c, d) hatching at different embryonic salinities (E_S) for twelve combinations of larval temperature (L_T) and salinity (L_S). Symbols represent each combination of factors per population. Data shown as mean values ± SE among larvae produced by different females (n = 5 or 3). Asterisks represent significant differences between populations for each combination of $E_S \times L_S \times L_T$.

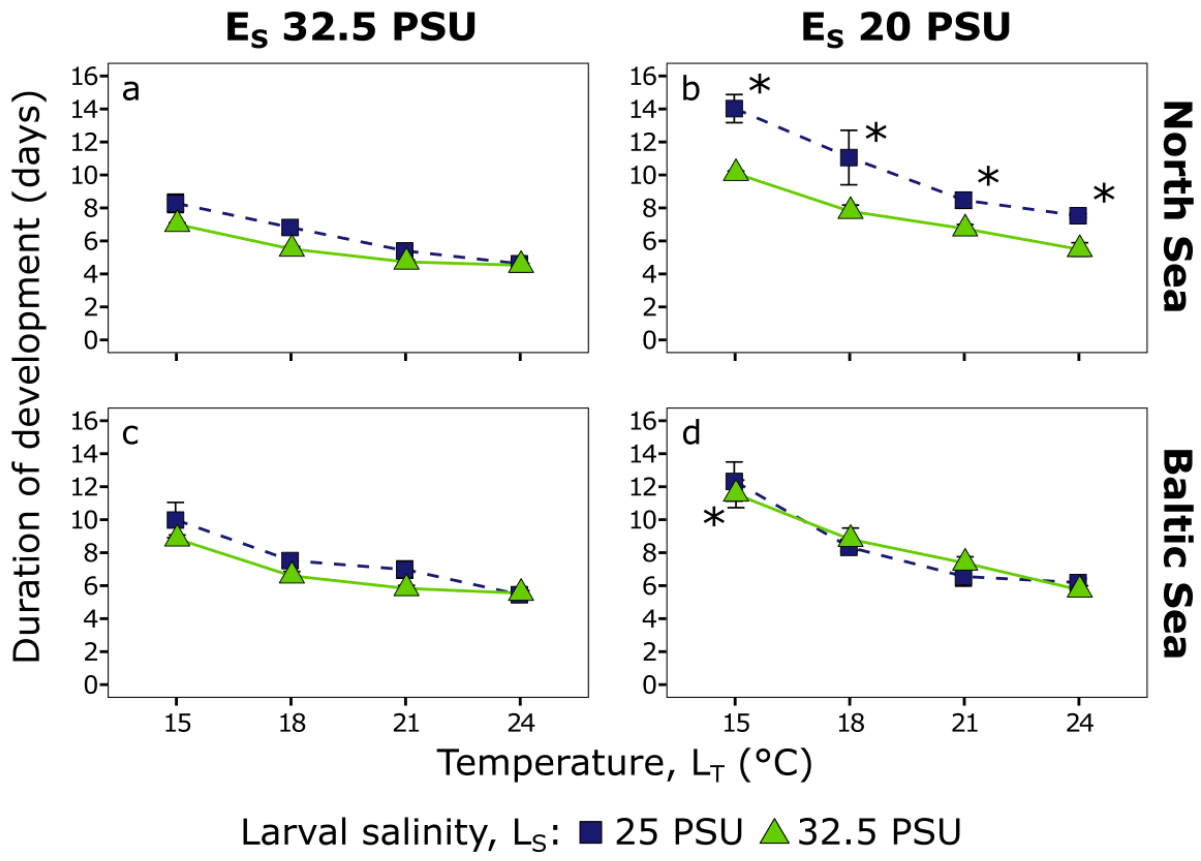

Figure 3. *Carcinus maenas.* Duration of larval development to zoea II. Comparison between populations hatching in different embryonic salinities, E_S (North Sea a, b; Baltic Sea: c, d) for eight combinations of larval temperature (L_T) and salinity (L_S). Symbols represent each combination of factors per population (note that L_S 20 PSU is excluded). Data shown as averages of developmental duration ± SE among larvae produced by different females (n = 5 or 3). Asterisks represent significant differences between populations for each combination of population × L_S × L_T.

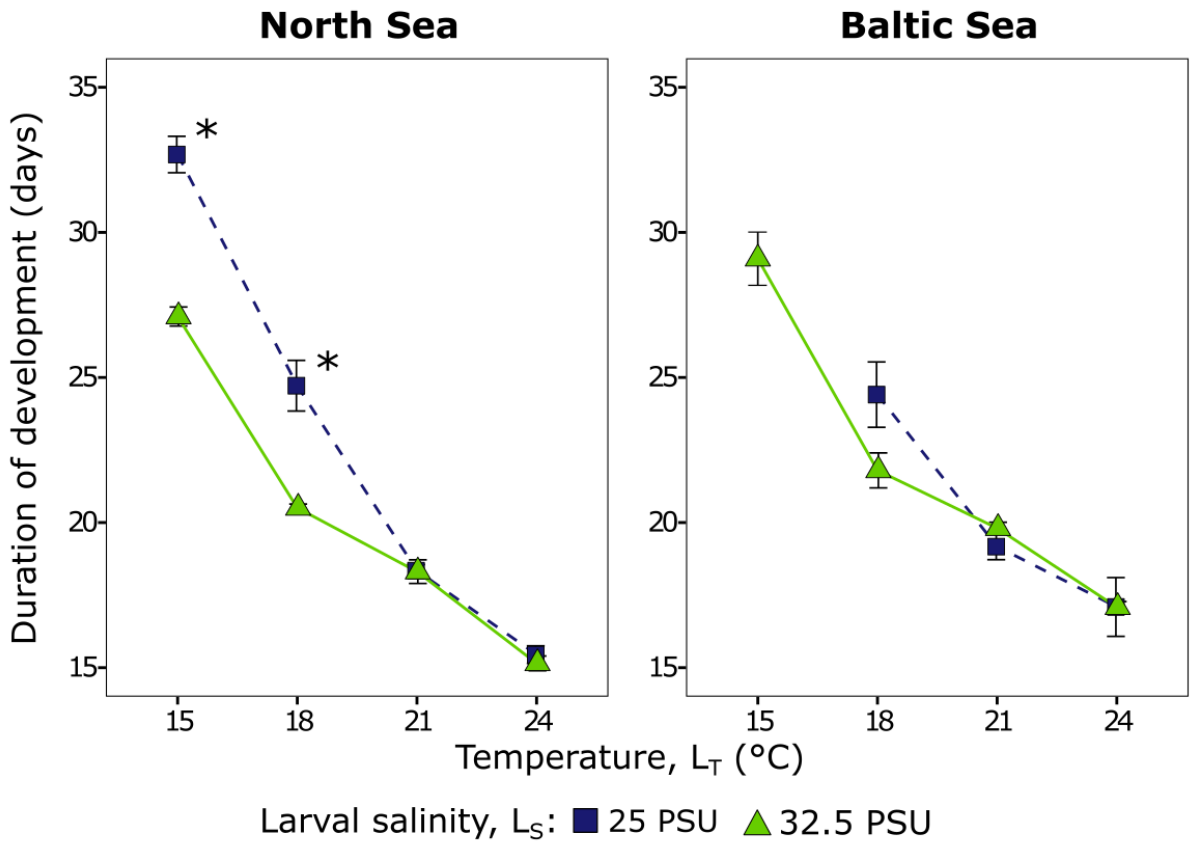

Figure 4. *Carcinus maenas.* Duration of larval development to megalopa from embryos reared at E_S = 32.5 PSU. Comparison between populations for eight combinations of larval temperature (L_T) and salinity (L_S). Symbols represent each combination of factors per population (note that L_S 20 PSU is excluded). Data shown as averages of developmental duration ± SE among larvae produced by different females (n = 5 or 3). Asterisks represent significant differences between larval salinities for each combination of population × L_T.

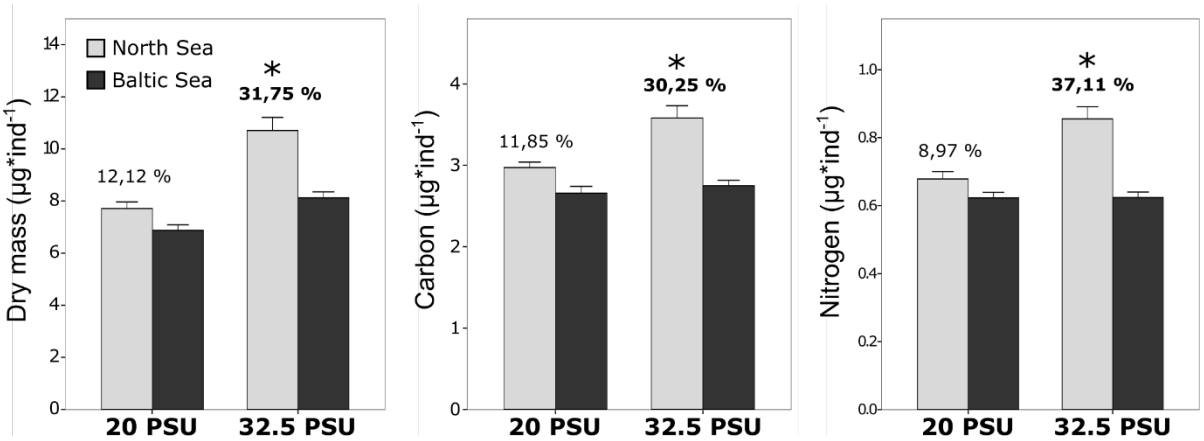

Figure 5. *Carcinus maenas*. Dry mass, carbon, and nitrogen content (µg*ind^{-1}) of freshly hatched zoea I after exposure to 20 or 32.5 PSU during embryonic development (E_S) from two populations (grey = North Sea; black = Baltic Sea). Bars represent mean values for each combination of E_S and population. Error bars represent ± SE among larvae produced by different females (n = 5 or 3). Percentages on the top of the bar represent significant differences between populations at each embryonic salinity. Asterisks represent significant differences between embryonic salinities (E_S).

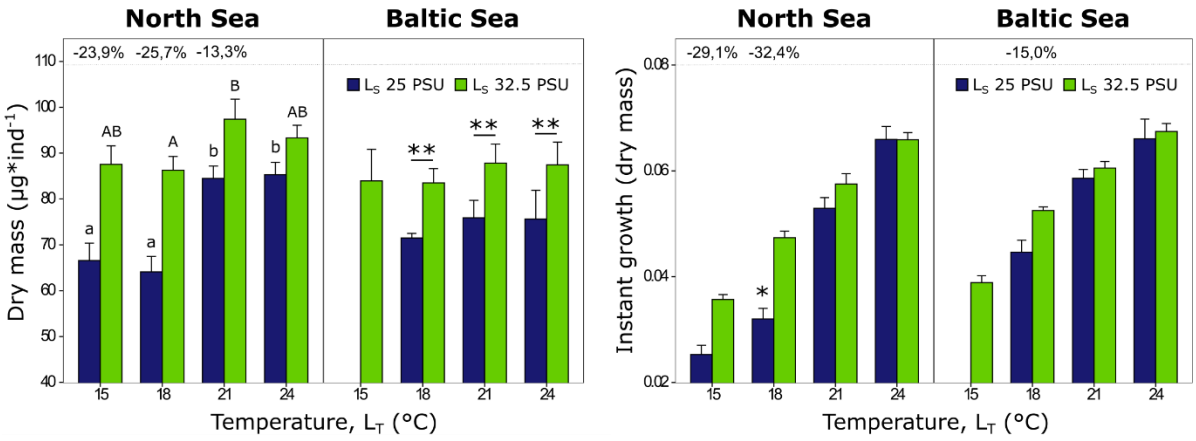

Figure 6. *Carcinus maenas*. Dry mass of megalopa and instantaneous growth rates (dry mass) from hatching to megalopa for two populations (North Sea *vs.* Baltic Sea) reared at two larval salinities (L_S: 32.5 PSU, green and 25 PSU, blue) and four temperatures (L_T: 15, 18, 21 and 24 °C). Data shown as average individual biomass (µg*ind^{-1}) ± SE and average individual growth (µg*ind^{-1}day^{-1}) ± SE among larvae produced by different females (n = 5 for North Sea, n = 3 for Baltic Sea). Different letters represent significant differences for total dry mass between temperature treatments (lowercased = L_S 25 PSU, capitalised = L_S 32.5 PSU). Percentage values on top of the panels represent significant differences between larval salinities at each temperature in each population. * show significant differences in the instant growth rates between populations for the same treatment. ** show significant differences between larval salinities in the Baltic Sea population (additional three-way ANOVA analysis of larval salinity excluding 15 °C).

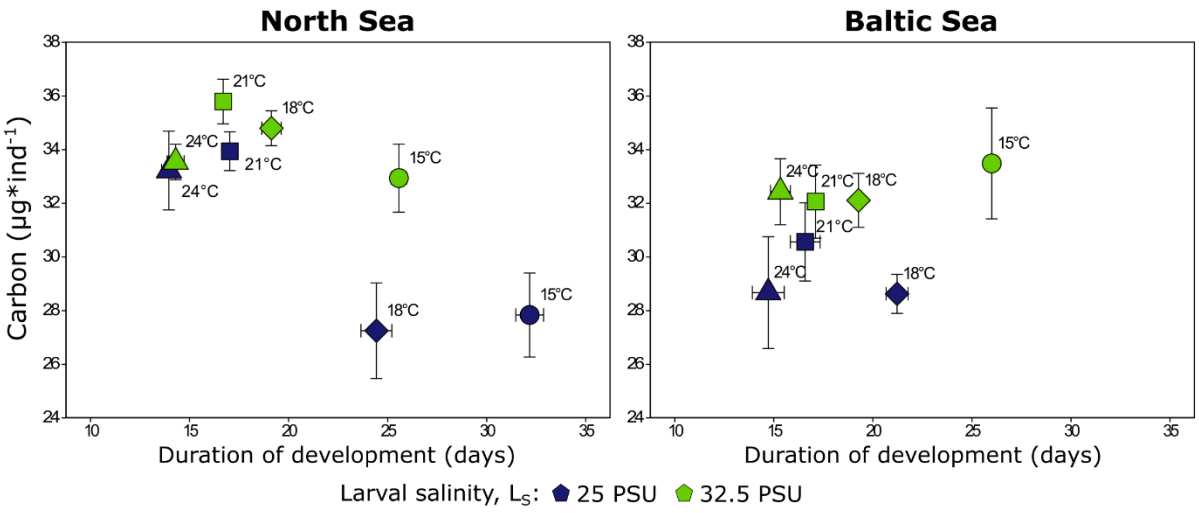

Figure 7. *Carcinus maenas*. Integrated responses of carbon content and developmental time for megalopa presented for two populations (North Sea = left panel *vs.* Baltic Sea = right panel) and different combination of larval temperatures, L_T (15 °C = circles, 18 °C = diamonds, 21 °C = squares, 24 °C = triangles) and larval salinities, L_S (25 PSU = blue, 32.5 PSU = green). Error bars represent ± SE among larvae produced by different females (n = 5 for North Sea, n = 3 for Baltic Sea).