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TEMPERATURE MODULATES COMPENSATORY RESPONSES TO FOOD LIMITATION AT METAMORPHOSIS IN A MARINE INVERTEBRATE

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ABSTRACT

1. Under climate change, increased temperatures combined with food limitation may be critical for species with complex life cycles, if high growth rates characterise the larval development.
2. We studied the effect of increased temperature and food limitation on larval survival and on functional traits (developmental time, body mass, growth) at moulting and metamorphosis in the crab *Carcinus maenas*
3. We followed the approach of models of metamorphosis integrating responses of body mass and developmental time to increased temperature and food limitation. We also evaluated if body mass decreased with temperature (according to the temperature-size rule) and if developmental time followed an inverse exponential reduction (expected from some metabolic theories), as both trends are relevant for modelling effects of climate change on fitness and population connectivity.
4. Larvae produced by four females were reared separately from hatching to metamorphosis to the megalopa at two food conditions (*ad libitum* and food limitation), and at four temperatures covering the range experienced in the field (<20°C) and those expected from climate change (>20°C).
5. In general, body mass did not decrease with temperature, nor developmental time followed an inverse exponential response to temperature (under *ad libitum* food conditions).
6. At low temperatures (<20°C), food limitation resulted (in general) in small reductions in larval survival. Body mass and nitrogen content were little affected by food limitation while effects on carbon content were small. Increased developmental time partially or fully compensated for reduced growth rates. We interpreted this response as adaptive, as minimising fitness costs associated to reduced body mass.
7. Increased temperatures (>20°C) exacerbated the effect of food limitation on mortality in larvae from three females. Developmental time was longer and larvae metamorphosed with reduced body mass, carbon and nitrogen content. Thus, compensatory responses failed and multiple fitness costs should be expected in individuals facing food limitation at increased temperatures.
8. We propose that integrative studies of traits at metamorphosis could be a basis to develop a mechanistic understanding of how species with complex life cycles will respond to climate change. Such models could eventually include hormonal and metabolic regulation of development as drivers of responses to environmental change.

Keywords: biomass growth, European shore crab, food limitation, growth rates, warming.

RESUMEN

1. Bajo condiciones de cambio climatico, la combinación de temperaturas elevadas y baja disponibilidad de alimento, podrían ser críticas para especies con ciclos de vida complejos si el desarrollo larval está caracterizado por tasas de crecimiento altas.
2. En este trabajo estudiamos el efecto de temperaturas elevadas y baja disponibilidad de alimento en la sobrevivencia larval y caracteres funcionales (tasa de desarrollo, masa corporal y crecimiento) durante la muda y metamorfosis del cangrejo *Carcinus maenas*.
3. En tal sentido, seguimos el enfoque de modelos de metamorfosis integrando respuestas de la masa corporal y tasa de desarrollo a temperaturas elevadas y baja disponibilidad de alimento. Además, evaluamos si la masa corporal disminuye con la temperatura (de acuerdo a la regla de temperatura-tamaño) y si el tiempo de desarrollo siguió una reducción exponencial inversa (esperada de algunas teorías metabólicas), ya que ambas tendencias son relevantes para modelar los efectos del cambio climático en el fitness y la conectividad de las poblaciones.
4. Larvas producidas por cuatro hembras fueron cultivadas por separado desde la eclosión hasta la metamorfosis a megalopa bajo dos condiciones de alimentación (*ad libitum* y baja disponibilidad), y cuatro temperaturas que cubren el rango experimentado en el campo (<20°C) y aquellas esperadas debido al cambio climático (>20°C).
5. En general, la masa corporal no disminuyó con la temperatura ni el tiempo de desarrollo siguió una exponencial inversa a la temperatura (bajo condiciones de alimentación *ad libitum*).
6. A bajas temperaturas (<20°C), la baja disponibilidad de alimento resultó (en general) en pequeñas reducciones de sobrevivencia larval. La masa corporal y el contenido de nitrógeno se vieron apenas afectados por la baja disponibilidad de alimento mientras que el efecto en el contenido de carbono fue pequeño. Un incremento en el tiempo de desarrollo compensó parcial- o totalmente a las tasas de crecimiento reducidas. A esta respuesta la hemos interpretado como adaptativa, minimizando los costos en fitness asociados a una masa corporal reducida.
7. Las temperaturas elevadas (>20°C) exacerbaron el efecto de la baja disponibilidad de alimento en la mortalidad de larvas provenientes de tres hembras. El tiempo de desarrollo fue más prolongado y las larvas metamorfosearon con un contenido reducido de la masa corporal, carbono y nitrógeno. Por lo tanto, las respuestas compensatorias fallaron y se deben esperar

múltiples costos en fitness en individuos enfrentando baja disponibilidad de alimento a temperaturas elevadas.

8. Finalmente, proponemos que estudios integrados de los rasgos en la metamorfosis podrían ser la base para desarrollar una comprensión mecanística de cómo especies con ciclos de vida complejos responderán al cambio climático. Dichos modelos podrían incluir eventualmente la regulación hormonal y metabólica del desarrollo como impulsores de respuestas al cambio ambiental.

INTRODUCTION

In species with complex life cycles, temperature can drive metabolic, growth and developmental rates, which in turn determine body size at metamorphosis (van der Have & De Jong 1996; Forster & Hirst 2012) and chances of survival and reproduction (Roff 1992; Stearns 1992). However, the effect of increased temperature on survival or traits of organisms should combine with that of additional environmental variables, leading to additive or interactive responses (Crain, Kroeker & Halpern 2008; Hoffmann & Sgro 2011; Piggott, Townsend, & Matthaei, 2015). In particular, food limitation can have important consequences on organisms when occurring at high temperatures. Increased temperatures may cause growth limitation by increasing metabolic demands and by shifting phenology and driving mismatches between the abundance of consumers and their prey (Edwards & Richardson 2004). Under optimal temperatures, organisms may respond to food limitation through various mechanisms (e.g. morphological changes in feeding systems: Reitzel & Heyland 2007; increasing feeding rates: Pochelon, Calado, Dos Santos, & Queiroga 2009; storing reserves at times when they access food and reducing activity when food is scarce: Anger 1987; Hood & Sterner 2010; Koussoroplis & Wacker 2016). However, under scenarios of increased temperature, the mechanisms compensating for food limitation may fail and the detrimental effects of food limitation on physiology and survival are likely to be exacerbated (Giebelhausen & Lampert 2001).

In a scenario of increased temperature, food limitation will be especially challenging at times when high growth and developmental rates demand access to large quantities of food over short time periods. Periods of high growth and developmental rates occur during the larval phase of many marine invertebrates and fish. For instance, within the ca. 5 weeks needed to complete the larval phase, marine crabs and lobsters increase 10 times their initial body masses (Anger 2001). Given that as much as 50% of the consumed resources can be lost only as respiration (e.g. see Anger & Harms 1989), high growth rates must be sustained by the consumption of a large amount of prey. It is therefore not striking to find that growth in the marine habitat is limited by natural levels of food availability (Olson & Olson 1989; Reitzel, Webb & Arellano 2004; Bos, Hendriks, Strasser, Dolmer & Kamermans 2006; Giménez 2010; Le Pape & Bonhommeau 2015).

In the aquatic environment, food limitation arises from the necessity to access food patches while minimising the risk of mortality by predation. Food items aggregate in patches

located towards surface waters where sunlight promotes photosynthesis. These patches can remain stable for days to weeks (Gallager, Yamazaki & Davis, 2004; Durham & Stocker 2011) and can occur at various spatial scales (Prairie, Sutherland, Nickols, & Kaltenberg, 2012). Patches of microalgae are found associated to discontinuities characterised by gradients in water density, such as pycnoclines, located across the water column or as fronts separating e.g. coastal and offshore water masses (Morgan, De Robertis & Zabel, 2005; Gómez-Gutiérrez, Martínez-Gómez & Robinson, 2007). Small-scale variations in growth or water density can generate thin layers of microalgae (Gallager et al. 2004; Durham & Stocker 2011). However, consumers may not remain inside food patches for long times; instead, many (e.g. jellyfish, copepods, larval stages of fish and crustaceans) perform diel vertical migrations, accessing food in surface waters during nigh time and migrating to deep waters during daytime. Diel vertical migration is considered the most important strategy to reduce risk of predation by visual predators (e.g. fish) at expenses of reducing food intake (Hays 2003; Liu, Sun, Han, 2003; Thygesen & Patterson 2019). An important consequence of diel vertical migration is that the access to prey patches is restricted to few hours every day. We know that some invertebrate larvae can successfully develop when access to prey is limited to 4-6 hours per day (Sulkin, Blanco, Chan & Bryant, 1998; Giménez & Anger, 2005; D' Urban Jackson, Torres & Giménez, 2014; González-Ortegón & Giménez, 2014). We have however little information about the consequences of increased temperature for the capacity to tolerate daily limitations in access to prey.

We use the European shore crab *Carcinus maenas* to study the effect of increased temperature on larval development under limited access to prey. *C. maenas* is native to Europe but it is one of the most successful marine invasive species worldwide (Roman & Palumbi 2004). *C. maenas* larvae exhibit diel vertical migrations (Dos Santos et al., 2008) and undergo large increases in body mass over the larval phase (>1000% from hatching to metamorphosis to the juvenile stage within 5-6 weeks: Dawirs, Püschel & Schorn, 1986). Larvae develop over a wide range of temperatures (12-25°C: Dawirs 1983). The larval phase occurs through four pelagic zoeal stages and is followed by a metamorphosis to the settling stage called megalopa; the megalopa will abandon life in the water column and invade (= settle on) shore habitats where it undergoes further metamorphosis into a juvenile crab stage (Spitzner et al., 2018).

We quantified the effects of limited access to prey and increased temperatures on larvae in terms of survival, developmental time, body mass, elemental carbon and nitrogen, and growth rates (i.e. the ratio of body mass accumulation and developmental time, e.g. see Forster,

Hirst & Atkinson, 2011). We were interested in achieving a more mechanistic understanding of how larvae deal with food limitation in a warming environment. We therefore based our analysis on studies of growth strategies (Gotthard 2001; 2004) and factors triggering metamorphosis in species with a complex life cycle (Smith-Gill & Berven 1979; Bradshaw & Johnson 1995; Hentschel 1999; Hentschel & Emlet 2000; Howard & Hentschel 2005). This framework recognises that body mass, developmental and growth rates are linked traits (Gotthard 2001; Shingleton 2011; Torres, Spitzner, Harzsch & Giménez 2019) and that trait changes do not reflect only passive stress responses, but instead may be adaptive (Gotthard & Nylin 1995) in the sense that they occur as a way to minimise fitness costs. In particular, body mass is expected to reflect trade-offs between growth and developmental rates (Werner 1988; Heyland, Degnan & Reitzel, 2011). Trade-offs occur because (if growth rate is constant) minimising costs of a longer development can be achieved only at expenses of increasing costs associated with reduced body mass. In marine invertebrates with a pelagic larval phase, the cost of reduced body mass consists of decreased post-metamorphic survival (Pechenik 2006; Chiu, Ng, Wang, Thiyagarajan & Qian 2007; Torres et al. 2016), while costs of a longer developmental time consist of increased risk of predation or longer exposure to stressors (Pechenik 1999). In seasonal habitats, metamorphosis must occur within an optimal time horizon (Gotthard, 2001). Shore crabs experience size-dependent cannibalism as juveniles (larger juveniles eat smaller ones: Moksnes, 2004) that could increase if individuals metamorphose with reduced body size or settle too late in the season. In addition, late settlement will expose early juveniles to low temperatures, reducing growth rates and possibly delaying maturation or reducing fecundity.

In order to better understand the outcome of our experiment, we considered several hypothetical scenarios of integrative responses of body mass and developmental time (Fig. 1). Following previous models of metamorphosis (Hentschel 1999), we expected a negative relationship between body mass and developmental time driven by effects of food limitation. In addition, one hypothetical thermal response of body mass consists of decreases with temperature, following the temperature-size rule (Atkinson 1994; Forster, Hirst & Atkinson 2011). Thus, the first scenario hypothesises that the combination of effects of food limitation and temperature should lead to a situation (Fig. 1, scenario 1) where larvae reach a "conditional" threshold of body mass set by temperature but food limitation would drive reductions in body mass and extensions of developmental time (as in an L-shaped reaction norm: Stearns & Koella 1986). For developmental time, we evaluated if the response to

200 temperature was consistent with expectations of the metabolic theory of ecology and hence
201 followed the so-called "universal temperature development relationship", UTD (i.e. an
202 exponential decrease in developmental time with the inverse of the absolute temperature:
203 O'Connor et al. 2007). If developmental times were explained by the UTD, and body mass was
204 to follow the expectations from models of metamorphosis and the temperature-size rule, we
205 would have a starting point for the development and further test of a general theory to predict
206 effects of food limitation and increased temperatures on the timing and body mass at
207 metamorphosis. In addition, we considered alternative scenarios because in crustacean larvae,
208 there are situations where suboptimal conditions result in lengthening of the larval phase,
209 leading to increased body mass at metamorphosis (Giménez & Torres, 2002) consistent with
210 the hypothesis that growth is prioritised over development (Knowlton, 1974). The alternative
211 scenarios differ in the degree of body mass compensation whereby reductions in growth rates,
212 caused by food limitation, are matched by increases in developmental times. Compensatory
213 responses should reflect the fact that the highest fitness costs are associated to traits that vary
214 less (Gotthard, 2001). Therefore, we consider scenario 2 (Fig. 1) as reflecting a strong tolerance
215 to food limitation. In scenario 3, the effect of food on body size is the same across temperatures
216 (scenario 3 is expected from the model of Hentschel 1999 at each temperature); in scenario 4
217 the effects of food limitation on body size is stronger at higher temperatures (it may be that full
218 comensation occurs at optimal temperatures). In addition, we expected to observe
219 compensatory responses whereby food limitation leads to stronger effects on carbon than
220 nitrogen content (carbon is a proxy for lipid reserves and nitrogen is a proxy for protein content:
221 Harms, Meyer-Harms, Dawirs &, Anger 1994). Such expectation is based on the fact that crab
222 larvae use lipids reserves rather than proteins in order to cope with suboptimal conditions
223 (Harms et al. 1994; Torres, Giménez & Anger 2011).

224

225 ***FIGURE 1***

226

227 **METHODS**

228 **Animal handling and experimental design**

229 *Carcinus maenas* berried females were collected in the intertidal of the island of
230 Helgoland (Germany) in spring of 2016. Females were kept in a temperature-controlled room
231 at 18°C in isolated aquaria with well oxygenated natural seawater (salinity = 32 PSU); water

and food was changed daily. Only females that produced hatches within 48h. of collection were used in order to avoid effects of acclimation to laboratory conditions.

Upon hatching, larvae of each female (total = 4 females) were sorted into 40 rearing vessels (500 ml) which were subsequently assigned to each of eight factorial combinations of two food levels of daily access to prey (6 or 24 hours per day) and four temperatures (15, 18, 21 and 24°C). Food was provided as freshly hatched *Artemia* sp. nauplii, at densities known to produce maximum survival (~5 nauplii/ml). Temperatures were controlled in climatic rooms; 15 and 18°C represent temperatures experienced in the German Bight during spring-summer (Wiltshire & Manly, 2004). Temperatures >20°C are expected as the consequence of steady warming due to climate change (1-3°C towards the end of century: Schrum et al. 2016) and as the consequence of the expected increment in the frequency of warm summers (Christidis, Jones & Stott, 2015). Larvae hatched form each female were reared in five replicates units (containers of 500 ml) with 50 freshly hatched zoea I each, under each of the eight experimental conditions. Larval rearing followed standard methods (Dawirs 1984, 1987; Spitzner, Giménez, Meth, Harzsch & Torres 2019). The experiment was initially set at 18°C and containers were transferred to the different rooms so that temperature would increase/decrease overnight to the desired levels to avoid thermal shock. Seawater and food were changed daily. For larvae under limited access to prey, food was available for 6 hours each day, between 10a.m and 4p.m. (cf. Giménez & Anger 2005). Containers were rinsed daily with hot tap water and seawater afterwards; live and moulted larvae were counted and recorded; dead larvae were recorded and discarded. This experiment was repeated four times for larvae produced by each of four females separately so that we could establish if responses varied due to maternal influences. Overall, the experiment was based on a total of 8000 larvae; we used 2000 larvae hatching from each female, assigned in groups of 50 individuals to each replicate vessel (2000 larvae per female =50 larvae x 5 replicates x 4 temperatures x 2 food conditions).

Body mass, carbon and nitrogen content were quantified in freshly hatched larvae and in post-moult zoea IV and megalopa (1 day after moulting). Samples for freshly hatched larvae (3 replicates, each of 50 zoea I) were taken separately from those used in the experiment (i.e. in addition of the 8000 cultured larvae). At other stages, samples (in triplicate) consisted in randomly taking 8 zoea IV or 2 megalopa respectively. Larvae were briefly rinsed with distilled water in order to remove salts; then blotted dry with filter paper, placed in pre-weighed Aluminium cartridges and stored at -20ºC for later analysis. Body mass was determined as dry weight after freeze-drying the samples for 48h. (Christ Alpha 1–4 freeze-drier), using a

265 microbalance (Sartorius SC2, nearest 0.0001-mg). Carbon and Nitrogen content were
266 determined from the same sample using an elemental Analyser (vario MICRO cube CHNS
267 analyser, Elementar Analysensysteme).

268 **Statistical analysis**

269 The experimental design (Fig. S1) contained 3-factors, crossed in a factorial design, with
270 two fixed factors, food condition and temperature, and female of origin as random factor. The
271 response variables were survival, duration of development, body mass and elemental
272 composition (C and N), and growth rate. For survival, developmental time, body mass and
273 elemental composition, the full statistical model was therefore a mixed model with a fixed part
274 based on food condition and temperature, and a random part with intercept and slopes
275 depending on the combination of female of origin, food condition and temperature. Survival
276 was analysed as proportions after logit transformation (Warton & Hui 2011). Proportions were
277 also analysed in logarithmic scale in order to test for the multiplicative effects as a null model.
278 The multiplicative model is the appropriate null model to evaluate if temperature and food
279 limitation operate independently on survival rates, according to the law of probabilities (Piggott
280 et al. 2015); such model cannot be tested in a straightforward manner if proportions are
281 expressed in a logistic scale (Supplement section, Methods: data transformation, Fig. S2).
282 Duration of development, body mass and elemental composition were analysed in the raw
283 scale.

284 For growth rates, the full model was fixed factorial (food condition and temperature as
285 factors); these rates were calculated using average values of dry mass or elemental composition
286 of larvae exposed to each treatment combination, taken from each female. There were therefore
287 four replicate values of growth rates per treatment combination, corresponding to larvae from
288 each of the four females. We calculated instantaneous growth rates, as $g = log(W_f/W_0)/T$. In
289 this formula W corresponds to the average dry mass, carbon or nitrogen content and T is the
290 average developmental time from hatching to zoea IV or megalopa: W_f is the corresponding
291 average value at the zoea IV or megalopa and W_0 is the average value at hatching.

292 Statistical analyses were carried out through generalised least squares, linear mixed or
293 fixed models (Zuur, Ieno, Walker, Savaliev & Smith 2009; Galecki & Burzykowski 2013), in
294 R (R core team 2013) using the nlme and gls functions of the package nlme (Pinheiro et al.,
295 2018). Both functions fit linear models by maximum likelihood (instead of minimum squares)
296 and can accommodate cases of variance heterogeneity, lack of independent of residuals. The

lme function can accommodate many scenarios combining fixed and random factors. All variables, except growth rates were analysed with female of origin as a random effect in the full model. The mixed full model did not contain co-variances between food condition, temperature and female of origin; the random part of the model was coded as *"random= list(ffem = pdDiag(~ffood*ftemp))"*, where *ffood*, *ftemp* and *ffem* correspond to food condition, temperature and female of origin as factors. There were smaller models containing random terms depending on food condition and temperature (e.g. as *"random= 1+ ffood|ffem"* or *"random= 1+ ftemp|ffem"*) or only random intercepts related to the female of origin (e.g. as *"random= 1|ffem"*); the latter was the minimal expression of the random part considered here (all models had a random term). In biological terms, the model considers sources of variation that will reflect genotype by environment interactions. There were few cases where the fit of the full model failed due to situations of a singular matrix, where we followed procedures outlined by Bolker et al. (2009, Box 4) and reduced the starting model. For growth rate, the full model (fitted through general least squares) contained terms for variance heterogeneity and included correlations associated to female of origin (as a repeated measures design), in order to take the dependence structure associated to the female of origin. For growth, repeated measures were controlled with the corCompSymm function.

The test of whether developmental time responded to temperature according to the universal temperature dependence model (UTD) was carried out through polynomial regression, using the orthogonal polynomial approach. According to the UTD (O'Connor et al. 2007), the duration of development (D) is predicted as $D = a \cdot e^{f}$ with $f=b/[k(T+273)]$, T = temperature in degrees Celsius (a is a constant, $b=0.64$ is the "activation energy" and $k=8.62x10^{-5}$ is the Boltzmann constant). We log transformed the data of duration of development so that we obtained $\log(D) = c_0 + c_1 \cdot f$. (*with* c_0 the intercept and c_1 the slope) as the null model; we refer to f as the "Arrhenius transform". An alternative option is that the UTD is not a good predictor of effects of temperature on developmental time; in that case the response should be non-linear. Here we used a quadratic function as an alternative model: $\log(D) = c_0 + c_1 \cdot f + c_2 \cdot f^2$. The models were run with two interacting covariates (food condition and f) and random terms were defined by the combination of the factor "female" and the covariates.

The effect of food condition and temperature on all response variables was evaluated using a combination of model selection and hypothesis testing approaches as follows. First, model selection was applied through the backwards approach (i.e. starting with the full model)

and ranking models through the corrected Akaike information criteria (AICc). When the simplest model had the lowest AICc, that model was selected (applying the principle of parsimony); if $\Delta AICc > 3$, the model with lower AICc was selected irrespective of differences in complexity. Hypothesis testing (likelihood ratio tests) was applied when $\Delta AICc < 3$, and the most complex model had the lower AICc. When models differed significantly ($p<0.05$) the one with lower AICc was selected; in the opposite situation, the principle of parsimony was applied and the model with lower number of parameters was selected. In the first step, we applied model selection to the random structure (based on restricted maximum likelihood fitting: REML) with the full fixed effects included in the model. In the second step, model selection was applied on the fixed structure (based on maximum likelihood: ML), with the best random structure obtained in step-1. The best model was the one containing both the best random and fixed structures.

RESULTS

Depending on the female of origin, 25-75% of the larvae reared under a limited period of access to prey reched the megalopa (Fig. 2, S3 upper panel). Food limitation reduced survival in larvae on average and for larvae of each female in particular (Fig. 2). When data were logistically transformed, the best model was interactive except for survival to the second zoeal stage and the megalopa (Table S1). In addition, the multiplicative model of independence was also rejected (Table S2; exception: survival to zoea II and megalopa). The magnitude of the effect observed as the average response, varied among larvae hatching from different females (see Fig. S4 for female by female data). Analyses by female of origin showed that synergistic pattern was found over the full range of temperatures in larvae from all females, except in those hatching from female 4 (F4). For F4, the effect of food was consistent across all temperatures. For the megalopa, the pattern (logistic transformed data) was dominated by synergistic (F1) and additive effects (F2 & F4): explaining why the average response of increased temperature and food limitation was a decreased survival (Fig. 2, see also Fig S2).

FIGURE 2

Average responses of body mass and elemental composition responded only to food limitation, or synergistically to food limitation and increased temperature (Fig. 3; Figs. S5-S6); evidence of synergistic effects were found in the nitrogen content of zoea IV and in dry mass and nitrogen content of the megalopa (Tables S3-S4). Responses to food limitation varied by female: for the zoea IV, larvae from most females showed consistent effects of food limitation in dry mass and carbon content ((Fig. S7: exception F2 with synergistic effects); responses in terms of nitrogen content were synergistic in three females (Exception: F4). For the megalopa, in three of the four females the effects on dry mass and carbon content were interactive and the strongest effects of food limitation occurred at the highest temperature (Fig. S8: exception F3); at 18 or 15°C the effects of food limitation were minimal and inconsistent among larvae from different females. For nitrogen content, the effect of food and temperature was interacting in larvae from all females.

FIGURE 3

Average developmental time increased under food limitation and decreased at higher temperatures (Fig. 3). Food limitation caused an increase in developmental time across temperatures already at the early stages (Fig. S3, lower panel), and the effect became stronger at the megalopa (Fig. 3, Table S5). Responses by female of origin showed that the effect of increased temperature and food limitation occurred over the full range of temperatures considered: such pattern was synergistic for all females (Fig S9). For F4, a the synergistic effect was observed in the range 18-24°C, as larvae moulted to advanced stages (e.g. zoea IV and megalopa), but the effect of food limitation was larger at 15°C (Fig. S9). Developmental time deviated from the pattern expected by the universal temperature dependence (UTD), under both *ad libitum* and food-limited conditions (Fig. S10, Table S6). In all cases, a quadratic model fitted better than the linear model expected from the UTD.

Calculations showed that average instantaneous growth rates responded synergistically to increased temperature and food limitation (Fig. 3; Fig. S5-6, Table S7); they increased with temperature under *ad libitum* conditions. Under food limitation, growth rates were consistently low.

On average, the integrated responses at the zoea IV (Fig. 4) were consistent with scenario 4 in Fig. 1, i.e. *ad libitum* fed larvae moulted at some maximum threshold of body mass (dry

mass, carbon and nitrogen content) and food-limited larvae reached a lower threshold of body mass conditional on temperature. In larvae from three of the four females (Fig. S11-S13) the upper threshold was achieved by *ad libitum* fed larvae irrespective of temperature (exception F1 where a lower threshold was reached at 21 and 24°C). In addition, in three out of the four females, the lower biomass was conditional on temperature as in the average response (except in F4 where it did not vary with temperature). Average responses at the megalopa were consistent with scenario 3 in Fig. 1 (Fig. 4): food-limited larvae, reared at the lowest temperature (15°C), also reached the maximum threshold of body mass; minimum thresholds of body masses (conditional on temperature) were reached at increased temperatures (Fig. 4). When reared under *ad libitum* conditions, the upper threshold of body mass, depended on female of origin (Fig S14-S16: e.g. higher in F1 as compared to F2-4); for each female the upper threshold depended on temperature (21 or 24°C). Under food limitation, high levels of body mass were reached, at either 15 or 18°C depending on female, but not at 24°C.

FIGURE 4

Differential responses of carbon and nitrogen content to food limitation and temperature are shown in the C:N ratio (Fig. 5). At the zoea IV stage, low C:N ratios occurred in response to food limitation in all but the highest temperature. This is because at 15-21°C the percent carbon decreased more than the percent nitrogen in response to food limitation; but at 24°C both carbon and nitrogen decreased in similar proportions (Fig. S17). At the megalopa, C:N ratios where comparable across temperatures, due to similar effects of food limitation on each element, except at 15°C where drops in percent carbon were much higher than those in nitrogen (Fig. S17).

FIGURE 5

DISCUSSION

We quantified the effects of food limitation and temperature, on survival and on the integrated changes in body mass, elemental composition (carbon and nitrogen content), developmental time and growth rate during the ontogeny and metamorphosis of the shore crab

Carcinus maenas. We repeated the experiment with larvae hatched from different females, in order to consider the fact that responses may vary among families (Carter, Ceballos-Osuna, Miller & Stillman 2013; Appelbaum, Pan, Hedgecock & Manahan 2014; Spitzner et al. 2019). While such variation may reflect maternal effects or genetic variation (Marshall, Bonduriansky & Bussière 2008; Uller, Nakagawa & English 2013), our experiment is not designed to quantify, for instance genetic variation, as we would require a larger number of families. We think instead that repetition of experiment is critical in order properly study responses of larvae to multiple environmental stressors (as it is in any other field of science). We discuss the responses as averaged as well as by family, because interactions arising from among different families, when important, challenge the interpretation of main effects.

Food and temperature effects

On average, we found that larval tolerance to food limitation is stronger at the lowest tested temperatures, where larval survival and fitness costs associated to body mass were minimised at the megalopa. With some variation among families, at temperatures characterising the larval season (<20°C, North Sea, German Bight: Wiltshire & Manly 2004), food-limited larvae were able to sustain growth and development with only small reductions in survival. This is consistent with results of a previous study on the same species (survival to zoea II: Giménez & Anger 2005) and with other studies showing that marine invertebrate larvae are able to withstand food limitation as long as they have access to food for a few hours per day (Sulkin et al. 1998; Giménez & Anger 2005; D' Urban et al. 2014; González-Ortegón & Giménez 2014). At low temperatures, the effects of food limitation on growth up to the megalopa appeared to be partially compensated by extended development in larvae from some of the tested females; in larvae from three females, *ad libitum* and food-limited larvae metamorphosed to the megalopa stage with similar body mass and nitrogen content. In addition, the smaller effects on nitrogen than carbon content suggest some level of protection of the protein pool at expenses of lipid reserves. This interpretation is consistent with the stronger reductions in lipids and carbon content rather than in proteins and nitrogen content reported in shore crab larvae reared under suboptimal diets (Harms et al. 1994).

In larvae from the four tested females, at increased temperatures (>20°C) development was successful but at the expense of decreased survival, extended development and reductions in overall body mass, carbon and nitrogen content. Reductions in body mass suggest that extended duration of development did not compensate for the effect of food limitation on growth rates; reductions in nitrogen content, suggest that increased metabolic demands were

not matched by reducing the rate of lipid accumulation. Hence, increased temperature may entail costs of reduced body mass in larvae experiencing our scheme of restricted access to prey.

Fitness costs of size at metamorphosis or developmental time may occur through decreased early post-metamorphic survival or through reductions in fecundity, but their importance can vary among species (Twombly and Tisch 2002; Pechenik 2006). The contribution of early post-metamorphic survival to fitness should decrease as the post-metamorphic period extends. Although many marine invertebrates have a long post-metamorphic period, several studies have shown that the highest rates of post-metamorphic mortality occurs within a short period just after metamorphosis (48 h. to 4 weeks: reviewed in Gosselin & Qian1997) and that a driver of early post-metamorphic mortality is the conditions experienced by larvae (Pechenik 2006). This is also valid for *C. maenas*, where early post-metamorphic survival is driven by size-dependent cannibalism (Moksnes 2004) and size at metamorphosis is driven by food conditions experienced in the larval phase (Giménez 2010). We still know little about the actual contribution of such rates to the overall survival chances, especially in species with long generation times (> 4 weeks); a recent experiment using barnacles as model (Torres et al. 2016) has shown however that the effect of larval food limitation on early post-metamorphic mortality rates can still be detected (as reduced barnacle density) ~ 9 months after metamorphosis. Body size at metamorphosis can be important for fecundity when organisms metamorphose at nearly the adult size (Twombly and Tisch 2002). There is still little information about such contribution for marine invertebrates with long post-metamorphic growth periods. Laboratory experiments have shown that differences in size at metamorphosis can be carried over for months during the period of juvenile growth (Giménez, Anger & Torres 2004; including *C. maenas*: Giménez 2010). In crabs, fecundity correlate with maternal size (Hines 1982) but we do not know if differences in body size, carried over to the adult stages, are sufficient to affect fecundity. We think that fitness costs of reduced size at metamorphosis or extended development may be manifested primarily as increases in early post-metamorphic mortality possible up to the time when individuals reach a refuge in size (ca. 5 mm carapace length).

Integrative trait responses

For the case of trait responses, our study followed the integrative approach implemented in studies of ecological models of metamorphosis (Hentschel 1999). In order to make sense of the large set and the complexity of the potential responses, we used a series of hypothetical

scenarios (Fig. 1) and larvae from most families followed scenarios 3 (zoea IV) or 4 (megalopa). However, our study should not be interpreted as a general test of the different scenarios. The scenarios are a guide to interpretation of intergrative reponses. The different scenarios highlight the integrative nature of the response because they cannot be identified by looking at responses of isolated traits. For instance, take scenario 2 (Fig.1), where a maximum body mass is achieved irrespective of temperature and food conditions: the only condition is that the effects on growth rates are cancelled out by the effects on development time. There are any number of possible combinations of growth rate and developmental time leading to such matching. In addition, the scenarios recognise that processes occurring at low levels of organization are integrated but their responses do not map one-to-one into the responses at high levels (De Laender 2018). For example, in scenario 2, an additive response of growth rate to temperature and food, would be matched by an appropriate antagonistic response of developmental time; neither the additive not the antagonistic response turn up into the response observed in body mass at metamorphosis (there is no "one-to-one mapping"). Third, the scenarios allow some insight into the mechanisms driving in responses. In scenario 2, an adaptive hypothetical mechanism is that development time is physiologically regulated, perhaps by hormonal level. Other scenarios involved different mechanisms: for instance, the temperature size rule (and the underpinning mechanisms), when operating on larvae, should lead to scenario 1; in the same line, predictions of metabolic theories could lead to specific responses. The scenarios highlight that integrative models of metamorphosis can provide a framework to advance our mechanistic understanding of the effect of climate change on species with complex life cycles.

Our analysis enabled us to make a preliminary picture of the integrated trait responses to increased temperatures and food limitation that can occur in larvae from *C. maenas* (Fig. 6), and gain some insights into the likely underlying mechanisms. Under *ad libitum* food conditions, larvae moulted and metamorphosed at some maximum threshold irrespective of temperature; we did not find evidence supporting the temperature-size rule setting a conditional upper threshold of body mass, except for larvae of one out of 4 females. In addition, shorter developmental times were matched by increased growth rates, resulting in larvae metamorphosing to some maximum threshold of body mass; reduced developmental time, occurring at increased temperatures, was not well explained by the UTD. Hence, for the observed responses, we cannot fully explain body mass compensation through the mechanisms underpinning the UTD or the temperature-size rule. However, increases in growth rate with

temperature were consistent with previous studies on *C. maenas* larvae (Dawirs et al. 1986); such response is not trivial because growth rates can respond adaptively to temperature (Gotthard, Nylin & Wiklund 2000).

Under food limitation, we found evidence (in larvae from 3 females) that *C. maenas* can compensate for effects of food limitation on body mass at the lowest temperature (15°C). This response is consistent with the hypothesis of a hierarchy of responses, prioritising growth over development (Knowlton 1974). The capacity to compensate appears to vary among larvae hatched from different females: body mass compensation was achieved at 18°C in larvae from two females. Compensation was particularly strong in terms of nitrogen content, suggesting a protection of protein reserves at the expense of reserve accumulation (Carbon content: lipids). However, under food limitation and higher temperatures, larvae metamorphosed with reduced body masses, in spite of increased developmental time; at 24°C compensation was not achieved in larvae from any of the four females. At high temperatures, the failure in the compensatory response can be expressed as a proportional reduction in body mass in spite of proportional increase in developmental time (summarised in Fig. 6). The fact that food-limited larvae would appear to follow the temperature-size rule is not trivial. For instance, Diamond & Kingsolver (2010) found that body mass of tobacco hornworm reared under food limitation and below optimal temperatures, followed the reverse of the temperature-size rule. It would be interesting to test if stronger reductions in body mass occur towards both the upper and lower limits of thermal tolerance, in combination with food limitation.

FIGURE 6

Because growth rates were maintained at some minimum threshold in food-limited larvae, it appears that compensation failed due to limitations in extending developmental time. Plasticity for developmental time is usually reduced by 70-80% of the total larval development (Howard & Hentschel 2005) and it is constrained within each stage to the early periods of the moult cycle (post-moult and intermoult Anger 2001, Chap. 4). Within the moult cycle, constraints are set by moulting hormones triggering the initiation of the "pre-moult" period, when development becomes food independent (Anger 2001, Chap. 4). Compensatory responses for developmental time can occur across zoeal stages and may explain why body mass compensation, observed at the megalopa, was still not achieved when larvae moulted to

554 zoea IV; perhaps larvae at such stage are more efficient in feeding over short time periods than
555 at earlier stages. Constraints in plasticity for developmental time may be avoided in the field
556 by extending the daily feeding periods. Such strategy would maintain high growth rates, but it
557 would imply an alteration of the timing of vertical migration. Extending the feeding period
558 would therefore come at the cost of increasing the risk of predation by visual predators (Hays
559 2003); predation risk constitutes one of the key costs associated to growth at the maximum
560 possible rate (Ludwig & Rowe 1990; Gotthard 2004). In any case, in scenarios of increased
561 temperatures (e.g. in warm summers), compensatory responses may not be sufficient to
562 minimise costs of developing under food limitation because of costs associated to either
563 reduced body mass (increased post-metamorphic mortality) or high growth rates (increased
564 larval mortality).

565 We have interpreted trait changes as a result from phenotypic plasticity, but selective
566 mortality offers an alternative interpretation (Hechtel & Juliano 1997). Food limitation may for
567 instance select individuals characterised by extended development, i.e. having sufficient time
568 to accumulate reserves to some minimum threshold. This second mechanism, if widespread in
569 a population, would result into a portfolio effect (Schindler, Armstrong & Reed 2015), i.e. a
570 compensatory response occurring at the level of a population, whereby selection for genotypes
571 with e.g. longer developmental times buffers a population from fluctuations in food
572 availability. We have two main reasons to think that the most likely mechanism is phenotypic
573 plasticity. First, the observed responses of developmental time to food limitation and
574 temperature occur under experimental conditions did not impact mortality rates: these include
575 effects of temperature on developmental time (in *C. maenas*: Dawirs 1987; in ectotherms in
576 general: Van der Have & De Jong, 1996; Forster & Hirst, 2012), effects of short starvation
577 periods (24 hs.) on developmental time and body mass (Dawirs 1984; Anger 1987) and effects
578 of limited access to prey (survival was >80%: Giménez & Anger 2005; D' Urban Jackson et
579 al. 2014). Second, selective mortality predicts a positive correlation between survival and
580 developmental time, but we found negative correlations (Fig. S18). Similar negative
581 correlations have been found in previous studies (Giménez & Anger 2003; Macpherson &
582 Raventos 2005) and may be driven by traits linked to developmental time (e.g. body size). We
583 suggest that food limitation can lead to both a plastic response and a selective effect, but the
584 latter is unlikely to have driven the treatment effects observed in our experiments.

585

586

Conclusions

Overall, our analysis highlights the importance of the integrative and mechanistic approach to predict biological responses to climate change. Integrating traits responses point towards hypothetical underpinning mechanisms, which may be tested in future studies. As discussed by others (Kroeker, Kordas & Harley 2017; De Laender 2018), a mechanistic approach is urgently needed. A better understanding of strategies for growth and metamorphosis can be the basis for models linking environmental change, trait responses and subsequent effects in terms of survival. Such approach would then consider both plasticity and selective mortality and the importance of compensatory responses during development and portfolio effects. In particular, models of metamorphosis (Hentschel 1999) may constitute the starting point towards the integration of regulation of development, bridging the gap between ecological developmental biology and climate change (Torres et al. 2019). In our case, we identified a limit in the compensatory effect of developmental time on body mass (reflecting a developmental constraint, or reduced genetic variation). Understanding the causes and consequences of such types of limits might help us to develop the so desired mechanistic understanding.

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Authors' contributions: GT performed the experiments. Both conceived the experiments, analysed the data, wrote the manuscript and gave final approval for publication.

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Data accessibility: The data sets that support this study are deposited in PANGAEA ®Data Publisher https://www.pangaea.de (PDI-24164, under review).

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854
855 **FIGURE LEGENDS**

856 **Figure 1.** Four hypothetical scenarios of integrated changes of body mass and developmental
857 time in response to food limitation and temperature. Each scenario is summarised in four plots
858 showing growth trajectories (upper plot) and each response separately (remaining three plots;
859 error bars are only for illustration). Scenario 1 reflects reductions in body mass at higher
860 temperatures and effects of food limitation in both mass and developmental time. Scenario 2:
861 full compensation, changes in growth rates are matched by concomitant changes in
862 developmental time. Scenario 3: compensation fails and extended developmental time does not
863 match the reductions in growth rates. Scenario 4: compensation fails under food limitation but
864 with a smaller magnitude at low temperatures.

865

866 **Figure 2.** *Carcinus maenas*. Effects of temperature and food limitation on cumulative survival
867 from hatching to zoea IV and megalopa. Data are shown as average values ± SE for all four
868 females (thick lines) and discriminated by each individual female (thin lines). Values
869 corresponding to *ad libitum* access to prey are shown in blue (average: full thick line and light
870 blue circles; discriminated by female: full thin line and dark circles). Values corresponding to
871 limited access to prey are shown in green (average: dashed thick line and light green squares;
872 discriminated by female: dashed thin line and crosses). Percentages on top of the symbols give
873 the percent difference in survival or developmental time between the treatments of *ad libitum*
874 (blue) and limited access to prey (green). In all conditions, differences between food treatments
875 were significant. Cumulative survival from hatching to zoea II and III is given in Fig. S3.

876

877 **Figure 3.** *Carcinus maenas*. Effects of temperature and food limitation on body mass,
878 cumulative developmental time and instantaneous growth rate from hatching to zoea IV and
879 megalopa. The body mass (upper panels) is represented as µg ind^{-1}, the cumulative
880 developmental time (mid panels) is shown in days and the instantaneous growth rate (lower
881 panels) in day^{-1}. Percentages on top or below the symbols give the percent difference (dry mass,
882 development time or instant growth) between the treatments *ad libitum* and limited access to

prey at each temperature, when the difference was significant. See Figs. S5-S6 for responses of Carbon and Nitrogen content. Symbols as in Fig. 2.

Figure 4. *Carcinus maenas*. Integrated responses of dry mass and developmental time under different temperatures, and food conditions. Symbols as follows: 15°C: triangles, 18°C: squares, 21°C: diamonds, 24°C: cycles; limited access to prey: light green (indicated as "-") and *ad libitum*: dark blue (indicated as "+"). Error bars represent SE among larvae produced by different females (n=4). See lower panels in Figs. S12-13 and S15-16.

Figure 5. *Carcinus maenas*. Effects of temperature and food limitation on C:N ratios of zoea IV and megalopa. Percentages on top of the symbols give the percent difference between the treatments of *ad libitum* food (reference) and limited access to prey. Negative percent differences for the megalopa occur because C:N ratios under food limitation are increased due to strong reductions in nitrogen content. Symbols as in Fig. 2.

Figure 6. *Carcinus maenas*. Summary of average responses to temperature and food limitation. Body mass and developmental time are standardised so that values under *ad libitum* food conditions at 15°C (Reference: blue square) represent the unit. Under low nutritional stress (food limitation: 6 hours access to food at 15°C - green circle), larvae reach the upper threshold of body mass at metamorphosis (horizontal dashed line) at expenses of extended developmental time. Under increased temperatures, the nutritional stress becomes higher due to increases in metabolic demands and larvae metamorphose at some threshold of reduced body mass set by temperature (18, 21 and 24°C: yellow, orange and red circles, respectively). The proportional effect of food limitation on developmental time is appreciated once time is standardised. The light blue area represents the range of values of standardised time for the threshold of compensation.