

Contrasting oceanographic conditions during larval development influence the benthic performance of a marine invertebrate with a bi-phasic life cycle

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ABSTRACT: The pelagic life and benthic life of marine invertebrates with bi-phasic life cycles have often been studied separately, with the assumption that metamorphosis deletes larval history. However, it is now recognized that larval history can condition the post-metamorphic performance of benthic juveniles and ultimately contribute to the shaping of adult population dynamics. In the present study, we evaluated late larval and early juvenile performance of the green crab *Carcinus maenas* in Ria de Aveiro (Portugal) over 4 consecutive larval supply events in 2013. Larvae collected from the plankton were kept in the laboratory without food and under controlled conditions. Following metamorphosis, juvenile crabs were either exposed to starvation or provided with food ad libitum (*Artemia nauplii*). Juveniles were stocked until they either died or reached the 5th juvenile crab instar. Results showed that larval size at metamorphosis shifted along supply events, with this being reflected in early benthic life. Overall, optimal growth conditions did not delete the larval history experienced by the megalopae (and their previous zoeal stages) collected from each supply event. We analysed the influence of upwelling index in larval and early juvenile performance using general additive models, with the upwelling index explaining a significant percentage of the deviance recorded. Overall, contrasting trophic conditions experienced during larval life promoted a variation in larval size at settlement, which was carried over into early benthic life. A unifying integrated framework is therefore needed to better understand the population dynamics of marine invertebrates with bi-phasic life cycles and the role played by latent effects.

KEY WORDS: *Carcinus maenas* · Carry-over effects · Complex life cycles · Megalopa · Phenotypic plasticity · Post-settlement performance · Upwelling

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INTRODUCTION

The majority of marine invertebrates display complex life cycles that involve a pelagic larval phase and an adult benthic (benthopelagic) phase that are linked by larval hatching and metamorphosis. Benthic populations are conditioned by the success of their offspring in the plankton (Marshall & Morgan

2011) and favourable post-metamorphic conditions (Gebauer et al. 1999). Among other factors, larval survival in the plankton can be influenced by food supply, temperature and predation (Thorson 1950, Morgan 1995). Shifts in food quantity and quality experienced during larval life are paramount for larval growth (Rumrill 1990) and can shape larval phenotypes in such a way that may condition their

settlement success and early benthic life (Pechenik 2006).

A number of studies addressing the effect of supply of marine invertebrate larval stages on settlement success have been performed in recent years (Moksnes & Wennhage 2001, Queiroga et al. 2006, Olague-Feliu et al. 2010) and have provided important insights into larval ecology and behavioural responses under particular oceanographic conditions (Giménez & Dick 2007, Domingues et al. 2011). Nevertheless, Burgess & Marshall (2011) revealed in their study that variations in larval phenotype can play a more important role in the regulation of marine populations than shifts in larval supply. Studies conducted on marine invertebrates in this sense have confirmed that larval history and nutritional status play a major role in survival and settlement performance, being propagated into early juvenile stages (Phillips 2002, Jarrett 2003). As metamorphosis is an energetically expensive process, higher nutrient levels and energetic reserves at settlement can positively influence early juvenile fitness (Phillips 2002, 2004, Jarrett 2003). It is therefore legitimate to assume that the quality of settling larvae can not only condition juvenile performance post-metamorphosis, but can also affect the dynamics of populations and communities (Phillips 2002, Cowen & Sponaugle 2009, Giménez 2010). To understand both pelagic and benthic life we must therefore employ an integrated framework that allows us to unravel the complexity of the mechanisms regulating the population dynamics of marine invertebrates with bi-phasic life-cycles.

Upwelling is an important oceanographic phenomenon in the eastern boundary shelves worldwide (Menge et al. 2003, Pfaff et al. 2011) and along the western coast of the Iberian Peninsula in particular (Villegas-Ríos et al. 2011, Álvarez et al. 2013). During the spring–summer months, northerly winds prevail along the shelf, allowing the upwelling of deep, cold water (Fiuza et al. 1998). This water mass is rich in nutrients, which, together with dissolved CO₂ and solar energy, are used by phytoplankton to produce organic compounds through photosynthesis. This process generates high levels of primary production and, consequently, leads to high biological diversity (Guisande & Harris 1995, Guisande et al. 2001, Huthnance et al. 2002, Santos et al. 2004). Spawning and hatching of many marine invertebrates occur during the upwelling season, thus suggesting that larvae may somehow benefit from such conditions during their pelagic life (Shanks & Eckert 2005, Morgan et al. 2009).

The green crab *Carcinus maenas* (L.), an invertebrate species with a bi-phasic life cycle, occupies both hard and soft bottoms in intertidal and shallow subtidal habitats located along the shores, and in the estuaries and coastal lagoons of the western margin of the Iberian Peninsula (D'Udekem d'Acoz 1999). Populations of *C. maenas* release their larvae during spring and early summer (Crothers 1967, Lyons et al. 2012), with newly hatched larvae being exported to shelf waters where they develop through 4 different larval stages (termed zoea) and moult to a fifth and last larval stage (the megalopa) that later reinvades estuarine or coastal waters, commonly from March to June (Queiroga et al. 2006, Domingues et al. 2011).

The relation between the supply of *C. maenas* megalopae and oceanographic conditions has been analysed in detail in different locations (Queiroga et al. 2006, Giménez & Dick 2007, Domingues et al. 2011), revealing that larval supply mechanisms may vary within and among years. A modelling approach performed by Marta-Almeida et al. (2006) suggested that upwelling events can enhance the retention of larvae on the inner shelf and favour settlement. This scenario was later confirmed in a field study performed by Morgan et al. (2009). Nonetheless, few studies have explored the relation between contrasting oceanographic conditions experienced during larval development and success during early benthic life (Giménez 2010).

In the present study, we evaluated time to metamorphosis and size of *C. maenas* megalopae (using the number of days required to perform their metamorphosis since invading the estuary and carapace size at metamorphosis, respectively, as a proxy). We also evaluated the number of days that these specimens can survive prior to metamorphosis and after metamorphosis under starvation, thus solely relying on the catabolism of all energetic reserves accumulated prior to their collection from the plankton. The performance of juvenile crabs (using the number of days required to reach the fifth juvenile crab instar (C5) as well as C5 size and weight as a proxy) was also evaluated during 4 consecutive supply events (SEs). The following 2 null hypotheses were tested: (1) larval size at metamorphosis, time to metamorphosis and survival time of the first crab instar under starvation do not shift over consecutive SEs; and (2) under standard laboratory conditions, the performance of early juvenile crab instars does not shift with the SE of the megalopa, nor with the upwelling conditions experienced during planktonic larval development.

MATERIALS AND METHODS

Collection of wild megalopae

Ria de Aveiro is a shallow coastal lagoon (111 km²) on the western coast of mainland Portugal with a semidiurnal tidal circulation regime of ca. 13 h (Pereira et al. 2000). This complex estuarine system, formed by 2 jetties, shallow channels and branches (with an average depth of 1 m), creates a favorable habitat for large populations of *Carcinus maenas*.

Wild megalopae of *C. maenas* were collected during 4 consecutive SEs at Costa Nova, Canal de Mira (40° 37' 17" N, 8° 44' 56" W) in Ria de Aveiro using 2 passive plankton nets as described by Queiroga et al. (2006). During each SE, the nets were deployed for 24 h at a constant depth of 0.1 m and with their entrances always facing the inlet of the estuary. Plankton held in the collector cup was gently rinsed and poured into a bucket filled with seawater and transported to the laboratory for analysis. The 4 SEs (SE-1 to 4) occurred between the months of April and May 2013. The dates of the SEs surveyed were as follows: SE-1, April 10; SE-2, April 24; SE-3, May 10; SE-4, May 29. Sampling days were numbered according to the Gregorian calendar as days of the year in order to simplify data visualization and posterior analysis (SE-1 = 100, SE-2 = 114, SE-3 = 130; SE-4 = 149).

Experimental design

During each SE, *C. maenas* megalopae were individually sorted from the collected plankton samples in the laboratory using a light table and a modified plastic pipette. The number of megalopae sampled in each SE was as follows: SE-1: 96, SE-2: 76, SE-3: 80 and SE-4: 78. All sorted larvae were kept in individual PVC containers (300 ml) with artificial seawater (prepared by mixing Tropic Marine ProReef salt [Tropic Marine] with purified freshwater from a reverse osmosis unit) that was fully renewed on a daily basis. Controlled conditions of temperature (18°C), salinity (35) and photoperiod (12:12 h, dark:light) were employed for stocking sorted megalopae from the 4 SEs. No food was supplied to stocked megalopae until metamorphosis, with the rationale for this procedure being the assumption that megalopae entering estuaries have already reached the nutritional threshold required to perform metamorphosis (see Rey et al. 2015). It must be highlighted that, based on preliminary experiments using this approach, the average time required to reach metamor-

phosis under starvation is ~4 d; this period is not sufficient for a significant depletion of internal energetic reserves to take place. Following metamorphosis, all crabs in the first juvenile instar (C1) were haphazardly separated in 2 treatments: exposed to starvation (S) and provided with food ad libitum (F). In the F-treatment all crabs were supplied with newly hatched *Artemia* nauplii (provided daily ad libitum). Specimens from S and F treatments were visually inspected every day for exuviae or dead specimens until they reached the juvenile crab instar C5. Complete water changes were also performed after visual inspection and before supplying newly hatched *Artemia* nauplii. All shed exoskeletons were preserved individually in an 8:2 solution of ethyl alcohol (97%) and glycerine for posterior morphometric analysis, with carapace length (CL, measured from the tip of the rostrum to the posterior end of the cephalotorax) being determined for megalopa, and carapace width (CW, measured between the first pair of lateral spines on the topside of the carapace) being determined for juvenile crab instars C1 and C5. CL and CW were determined to the nearest 0.01 mm using a stereomicroscope (SZX16, Olympus) with a calibrated micrometer eyepiece. CW and wet weight (WW, to the nearest 0.01 mg) were recorded for all specimens reaching C5.

Statistical analysis

One-way ANOVAs were performed to compare time to metamorphosis and size of megalopae (CL), using SE (4 levels: SE-1, SE-2, SE-3, SE-4) as a fixed factor. The effect of larval size (i.e. CL) on survival time of the first juvenile crab C1 starved of food (S-treatment) was tested over consecutive SEs using ANCOVA, where larval size was used as the covariate. Linear relationship between the covariate and the response and homogeneity of regression slopes was confirmed. Post hoc Tukey's HSD test was used to investigate significant differences between SEs.

The early juvenile performance of specimens provided with food (F-treatment) was analysed using 1-way ANOVAs. In this way, number of days post-metamorphosis required to reach C5, CW of C1 and C5, and WW displayed by C5 were compared over consecutive SEs, using SE (4 levels: SE-1, SE-2, SE-3, SE-4) as a fixed factor. Post hoc Tukey's HSD test was used when ANOVA results revealed significant differences ($p < 0.05$). Assumptions of normality and homogeneity of variance were verified prior to analysis through Shapiro-Wilks and Levene's tests, res-

pectively. Whenever these assumptions were not verified, we employed power transformations before performing statistical analysis. The level of statistical significance was $p < 0.05$.

General additive models (GAMs) allow the exploration of non-linear functional relationships between dependent and explanatory variables, fitting predictor variables by smooth functions (Guisan et al. 2002) rather than by linear or quadratic relationships (characteristics of the linear models). GAMs were applied based on the R-mgcv function (Zuur et al. 2007). Our models introduced the upwelling index from the Aveiro region (41°N, 10.5°W; 41.5°N, 10°W; 41°N, 9.5°W; 40.5°N, -10°W) as an explanatory variable (data from Instituto Español de Oceanografía, www.indicedeafloramiento.ieo.es, methodology used as described by González-Nuevo et al. 2014). The rationale for this procedure was supported by available data from previous field (e.g. Queiroga 1996) and modelling studies (Marta-Almeida et al. 2006), which evidenced that upwelling favours the retention of larvae <20 km offshore in the study area. Settled megalopae in Ria de Aveiro can originate from larvae released by adult populations in southern (<60 km) or northern estuaries (<200 km) (Domingues et al. 2012), thus their larval development occurs off the north-western coast of the Iberian Peninsula. Assuming the upwelling index is quite homogeneous in this region (Haynes et al. 1993, Ramos et al. 2013), it is legitimate to assume that the upwelling index in the Aveiro region is a proxy for the upwelling index experienced by settled megalopae during their larval development. In this way, daily values of the upwelling index were condensed to 60, 50, 40, 30, 20 and 10 d averages before each SE. The maximum period (60 d) was selected based on to available estimates for the maximum duration of *C. maenas* planktonic larval development under water temperatures ranging between 12 and 15°C (see Dawirs 1985). One GAM was built for each response variable in each time interval (60, 50, 40, 30, 20 and 10 d). All values of upwelling indices were tested in the respective model to find the number of days that provided the best deviance explained by the response variables: CL, survival time, CW of C1, CW of C5 and WW of C5. Normality and outliers were checked following Zuur et al. (2007). The

effects of upwelling index were tested using the following equation:

$$E(Y_i) = \alpha + f_1(X_i) + \varepsilon \quad (1)$$

where $E(Y_i)$ is the estimated value of the response variable (CL, survival time, CW of C1, CW of C5 or WW of C5), α is the intercept, X_i is the covariate (upwelling index), f_1 is the smooth function estimated for the covariate, ε is the error term and i represents the observed data (Wood 2006). The following model was used to test for the CL of megalopae: $Y = \text{CL (mm)}$ and $X_i = \text{upwelling index (m}^3 \text{ s}^{-1} \text{ km}^{-1}\text{)}$. The following models were used to test for the different features recorded for juvenile crabs: $Y = \text{survival time for C1 (d)}$; $Y = \text{CW for C1 (mm)}$; $Y = \text{CW for C5 (mm)}$ or $Y = \text{WW for C5 (mg)}$ and $X_i = \text{upwelling index (m}^3 \text{ s}^{-1} \text{ km}^{-1}\text{)}$.

All data analyses were performed following the statistical package R (v.2.13.2) (R Development Core Team 2011).

RESULTS

In SE-1, SE-3 and SE-4, 100% of the megalopae were able to successfully metamorphose to C1, in SE-2, survival reached 97.4% (Table 1). The differences in time to metamorphosis were not statistically significant ($p = 0.1958$) between events (Table 1), although specimens from SE-1 displayed a longer time to metamorphosis than those from other events. Moreover, significant differences were recorded in the CL of megalopae supplied during different events ($p < 0.0001$), with those from SE-1 displaying the smallest CL (Fig. 1A).

When crabs were starved (S-treatment), survival time displayed by first juvenile instars increased with

Table 1. Summary of the experimental design employed to survey wild megalopae (M) of *Carcinus maenas* and juvenile crab stages exposed to starvation (S-treatment) or provided with food (F-treatment). Data refer to 4 consecutive supply events (SE), with the following variables being monitored: time to metamorphosis (mean \pm SD), survival from M to juvenile crab 1 (C1), survival time of C1 (mean \pm SD) and survival from M to juvenile crab 5 (C5). Different superscript letters represent significant differences among groups (Tukey's HSD post hoc analysis, $p < 0.05$)

Supply events	Day of year	Larval stage			Juvenile crab stages			
		Time to metamorphosis (d)	Survival (%) M-C1	n	S-treatment Survival time (d) C1	n	F-treatment Survival (%) M-C5	n
SE-1	100	3.7 \pm 1.1 ^a	100.0	96	6.5 \pm 1.5 ^a	56	95.0	40
SE-2	114	3.5 \pm 1.6 ^a	97.4	76	9.4 \pm 2.2 ^b	36	78.9	38
SE-3	130	3.5 \pm 1.0 ^a	100.0	80	12.4 \pm 1.7 ^c	40	90.0	40
SE-4	149	3.3 \pm 1.9 ^a	100.0	78	12.3 \pm 2.4 ^c	39	97.5	39

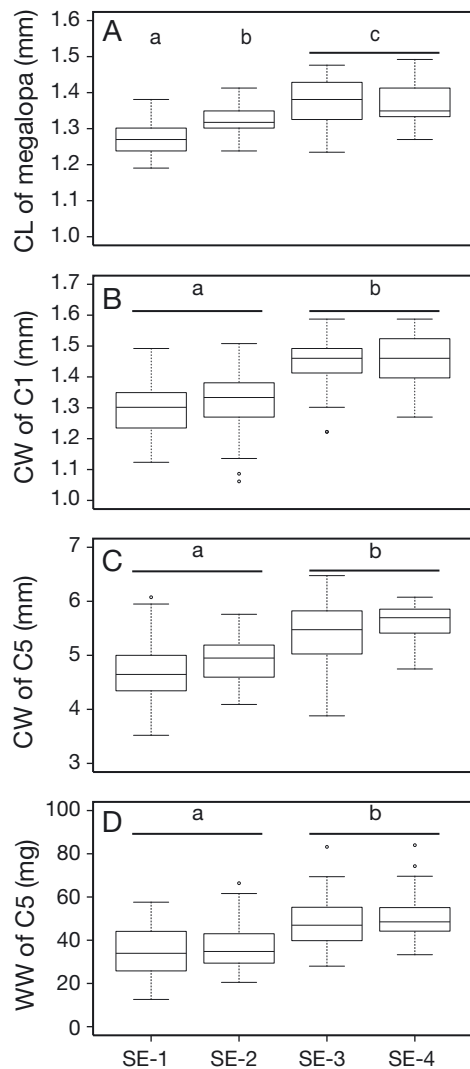


Fig. 1. Larval size at metamorphosis and early juvenile performance of *Carcinus maenas* during 4 consecutive supply events (SE, day of year): SE-1, 100; SE-2, 114; SE-3, 130; SE-4, 149. (A) Carapace length (CL) of the megalopa; (B) carapace width (CW) of first juvenile crab instar (C1) exposed to starvation (S-treatment) and provided with food ad libitum (F-treatment); (C) CW of fifth juvenile crab instar (C5) in F-treatment; (D) wet weight (WW) of C5 in F-treatment. The line within each box indicates the median, and 50 % of the values are within the box, while error bars represent range (from the minimum to the maximum value) and dots are outliers. Different letters represent significant differences among groups (Tukey's HSD post hoc analysis, $p < 0.05$)

days post-metamorphosis required to reach C5 was significantly different between events ($p < 0.0001$), with the lowest average number of days post-metamorphosis in SE-1 (Table 2).

Statistical analysis of CW of C1 revealed significant differences between SEs ($p < 0.0001$), with specimens from SE-1 and SE-2 displaying smaller CW than those from SE-3 and SE-4 (Fig. 1B). Analysis of CW of C5 also showed significant differences between SEs ($p < 0.0001$). Similar to C1, C5 from SE-1 and SE-2 displayed smaller CW than those from specimens originating from megalopae from SE-3 and SE-4 (Fig. 1C). WW of C5 also showed significant differences between events ($p < 0.0001$) and a similar trend (Fig. 1D).

In SE-1, SE-2 and SE-4, C1 was the instar with the highest mortality, with a number of cultured specimens being unable to reach C2, with the exception of SE-3, where all C1 reached stage C2. Moreover, the duration exhibited by each juvenile crab instar (Table 2) was significantly different between events (all at least $p < 0.0300$).

Upwelling regimes prevailing during larval development in the open ocean (Fig. 2) appeared to be significantly linked with the larval and juvenile per-

SE (Table 1). ANCOVA revealed a significant interaction between survival time and larval size (i.e. CL) ($R^2 = 0.59$, $F_{4,94} = 33.77$, $p < 0.0001$). Post hoc analysis showed significant differences ($p < 0.001$) between SEs (Table 1) (in other words, the larger the megalopae the longer the period endured under starvation by C1), with crabs originating from megalopae collected during SE-1 presenting the lowest CL and survival time (Fig. 1A and Table 1, respectively). In the S-treatment, none of the juvenile crabs C1 were able to moult to juvenile crab C2.

When crabs were supplied with food (F-treatment), at least 90.0 % of specimens from SE-1, SE-3 and SE-4 were able to reach C5, and 78.9 % from SE-2 (Table 1). The number of

Table 2. Stage duration (mean \pm SD) of juvenile crabs provided with food (Food-treatment) from juvenile crab 1 (C1) until juvenile crab 5 (C5), and days post-metamorphosis from C1 to C5, during 4 consecutive supply events (SE). Significant differences between supply events are represented by different superscript letters within the same column (Tukey's HSD post hoc analysis, $p < 0.05$)

Supply events	Day of year	Stage duration (d)				Days post-metamorphosis C1-C5	n
		C1	C2	C3	C4		
SE-1	100	4.2 \pm 0.6 ^a	4.2 \pm 0.8 ^a	5.2 \pm 2.1 ^a	9.2 \pm 1.3 ^a	23.8 \pm 3.0 ^a	40
SE-2	114	5.4 \pm 0.9 ^b	6.0 \pm 0.9 ^b	6.6 \pm 1.2 ^b	8.2 \pm 1.2 ^b	27.1 \pm 2.0 ^b	38
SE-3	130	5.2 \pm 0.7 ^{ab}	5.1 \pm 0.4 ^a	5.6 \pm 1.4 ^{ab}	8.6 \pm 1.4 ^{ab}	25.5 \pm 2.2 ^b	40
SE-4	149	4.8 \pm 0.8 ^{ab}	5.5 \pm 0.7 ^{ab}	6.5 \pm 0.9 ^b	8.2 \pm 2.0 ^{ab}	26.1 \pm 3.0 ^b	39

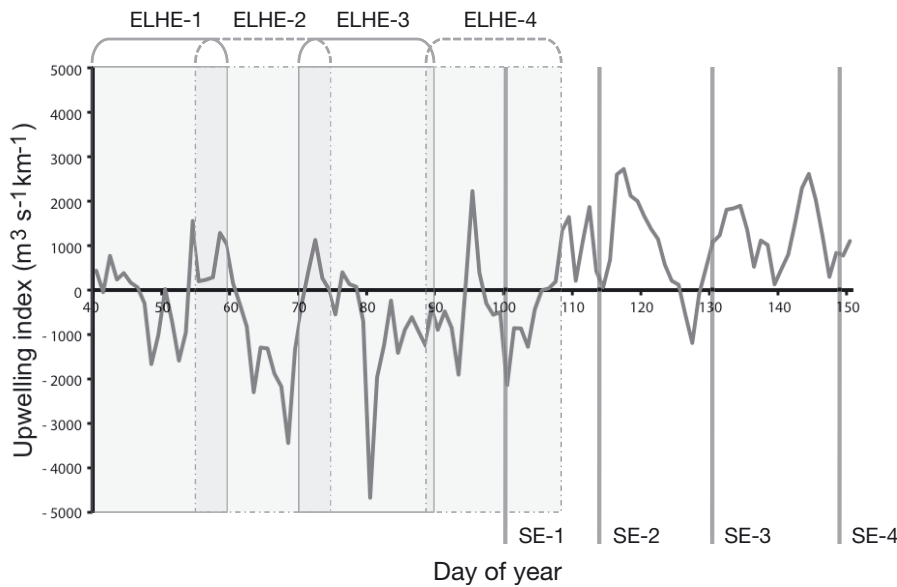


Fig. 2. Time series of the upwelling index in the coastal region of Aveiro, between Days 40 and 150 of 2013. Shaded areas inside each rectangle indicate the range estimated for larval hatching events (ELHE) and vertical solid lines indicate the day of year of each supply event (SE) surveyed for *Carcinus maenas* megalopae performance: SE-1, 100 (ELHE-1: 40–60); SE-2, 114 (ELHE-2: 54–74); SE-3, 130 (ELHE-3: 70–90); SE-4, 149 (ELHE-4: 89–109)

formance recorded in the present study (Table 3). According to the GAM for megalopae size (i.e. CL), the additive model using upwelling index as the explanatory variable indicated that CL presented a maximum value with the upwelling regime averaging around 500 m³ s⁻¹ km⁻¹, showing the highest deviance explained (26.2%) with an upwelling index average of 40 d before settlement (adjusted R² = 0.254, p < 0.0001) (Fig. 3A). Concerning GAMs for early juvenile performance, the smoothing function revealed that survival time displayed a positive correlation, showing the highest deviance explained (57.3%) with an upwelling index average of 30 d before settlement (adjusted R² = 0.564, p < 0.0001)

(Fig. 3B). The maximum survival time was achieved with upwelling regimes of around 600 m³ s⁻¹ km⁻¹. Moreover, CW of C1 achieved a maximum level with the upwelling regime averaging around 400 m³ s⁻¹ km⁻¹, showing the highest deviance explained (54.8%) with an upwelling index average of 40 d before settlement (adjusted R² = 0.536, p < 0.0001) (Fig. 3C). Regarding the F-treatment, the analysis of CW of C5 with smoothing function showed a maximum level with the upwelling regime averaging around 300 m³ s⁻¹ km⁻¹, with the highest deviance explained (35.3%) referring to an upwelling index average of 50 d before settlement (adjusted R² = 0.335, p < 0.0001) (Fig. 3D). The GAM analysis for

Table 3. Structure of the general additive models (GAMs) selected to describe larval size (carapace length [CL] of megalopae) and juvenile performance (survival time [ST], carapace width [CW] of crab instars 1 and 5 (C1 and C5) and wet weight [WW] of C5) of *Carcinus maenas* using the upwelling index (UI) (number of days of UI average that best explained the response variable) as explanatory variable. edf: estimated degrees of freedom

Parametric coefficients					Smooth terms (non-parametric)					
Parameter	Estimate	Standard error	t	p	Parameter	edf	F	p	R ² adjusted	% Deviance explained
CL										
Intercept	1.342	0.004	308.4	<2 × 10 ⁻¹⁶	UI (D40)	1.939	27.92	3.63 × 10 ⁻¹¹	0.254	26.2
ST										
Intercept	10.281	0.218	47.19	<2 × 10 ⁻¹⁶	UI (D30)	1.946	60.44	<2 × 10 ⁻¹⁶	0.564	57.3
CW – C1										
Intercept	1.379	0.009	160.3	<2 × 10 ⁻¹⁶	UI (D40)	1.971	41.17	1.52 × 10 ⁻¹²	0.536	54.8
CW – C5										
Intercept	5.227	0.058	89.61	<2 × 10 ⁻¹⁶	UI (D50)	1.922	17.74	6.00 × 10 ⁻⁷	0.335	35.3
WW – C5										
Intercept	43.115	1.356	31.79	<2 × 10 ⁻¹⁶	UI (D50)	1.931	11.92	3.55 × 10 ⁻⁵	0.251	27.1

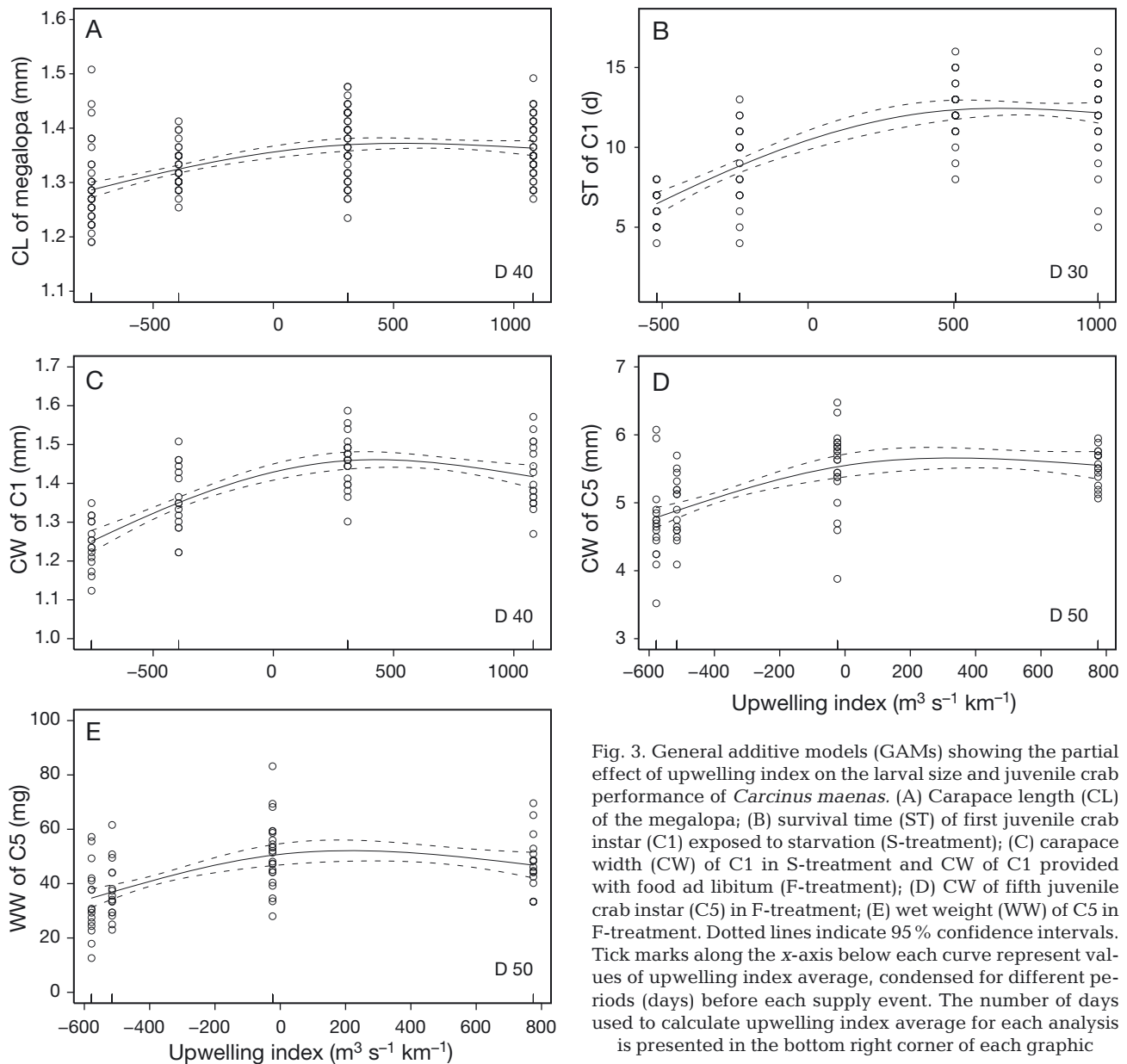


Fig. 3. General additive models (GAMs) showing the partial effect of upwelling index on the larval size and juvenile crab performance of *Carcinus maenas*. (A) Carapace length (CL) of the megalopa; (B) survival time (ST) of first juvenile crab instar (C1) exposed to starvation (S-treatment); (C) carapace width (CW) of C1 in S-treatment and CW of C1 provided with food ad libitum (F-treatment); (D) CW of fifth juvenile crab instar (C5) in F-treatment; (E) wet weight (WW) of C5 in F-treatment. Dotted lines indicate 95 % confidence intervals. Tick marks along the x-axis below each curve represent values of upwelling index average, condensed for different periods (days) before each supply event. The number of days used to calculate upwelling index average for each analysis is presented in the bottom right corner of each graphic

WW of C5 showed a maximum level with the upwelling regimen averaging around $200 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$, showing the highest deviance explained (27.1%) with an upwelling index average of 50 d before settlement (adjusted $R^2 = 0.251$, $p < 0.0001$) (Fig. 3E).

DISCUSSION

The results of the present study allow us to reject our first null hypothesis, as larval size at metamorphosis and survival time of the first crab instar under starvation significantly shifted over consecu-

tive SEs, although time to metamorphosis did not display any significant differences between events. The large number of megalopae collected from the plankton that were able to metamorphose to C1 under starvation conditions suggests that the last larval stage of *Carcinus maenas* had already reached the point of reserve saturation (PRS, when fed larvae attain the capability to develop through the rest of the moulting cycle using energy reserves already stored, sensu Anger & Dawirs 1981) before entering the coastal lagoon. Furthermore, the short time frame recorded between the time of entrance in the coastal lagoon and metamorphosis suggests that the majority of

megalopae collected were already in an advanced stage of their intermoult cycle, as already suggested by González-Gordillo et al. (2004) and Rey et al. (2015).

Megalopae sampled during the present study exhibited an increase in their CL along supply events. Indeed, the larger size displayed by the last larval stage of *C. maenas* collected during the last 2 events (SE-3 and SE-4) suggests that these had developed under more favourable environmental and trophic conditions than conspecifics supplied during early events. Our data contrasts with that reported by Giménez (2010), who detected a decrease in the size of the megalopae supplied during the settlement season in Helgoland (North Sea, German Bight). Larger megalopae commonly give origin to larger-sized juvenile crabs, which display a superior performance during their early benthic life (Giménez 2010). These opposing trends in the size of the megalopae supplied during the settlement season of *C. maenas* are most probably linked to the contrasting oceanographic regimes, and consequently trophic ecology, experienced by larvae developing in the Western Iberian upwelling system (Fiuza et al. 1998) and those developing in the German Bight (Giménez & Dick 2007).

Results recorded for C1 in the S-treatment confirmed that the juvenile crab instar C1 is unable to moult to C2 without the energetic provisioning secured through exogenous feeding, as already reported by Adelung (1971). Under starvation, megalopae that have reached their PRS use up their energetic reserves to undergo the dramatic shifts associated with metamorphosis; this energetic and nutritional drain inhibits subsequent moulting and ultimately leads to the death of the juvenile crab if deprived of food (Hartnoll 2001).

The differences in the survival time of juvenile C1 between SEs contribute to the idea presented above that suggests that larvae settled along SE-3 and SE-4 may have spent their planktonic life under more favourable nutritional conditions than their conspecifics settling during SE-1 and SE-2. It must be highlighted that in our study a time frame of solely 30 d (the time elapsed between SE-1 and SE-3) was enough to duplicate the number of days that C1 were able to endure under starvation. The explanation for this contrasting performance may be related to the upwelling index recorded during the pelagic life of the zoal stages that gave origin to the megalopae collected during SE-1 and SE-3. Upwelling is an environmental index used to estimate coastal productivity (Tenore et al. 1995, Barth et al. 2007). Up-

welling conditions along the Portuguese west coast give origin to nutrient-rich waters that promote the occurrence of high levels of phytoplankton (Oliveira et al. 2009), which triggers bottom up effects along the planktonic trophic webs in the pelagic environment and support high biological productivity. This oceanographic phenomenon may lead to an increase in nutrients for planktonic organisms (e.g. planktonic larvae) and increase their settlement success (Blanton et al. 1987, Menge et al. 2003, Peteiro et al. 2011). The time series of the upwelling index (Fig. 2) shows that megalopae which settled during SE-3 and SE-4 spent their larval life under positive and intermittent upwelling regimens. The succession of active upwelling (water fertilization) and relaxation periods (onshore transport) favours plankton blooms, as well as coastal retention of primary and secondary production (Villegas-Ríos et al. 2011). As larval experience influences the post-settlement performance of marine invertebrates with complex life cycles (Pechenik 2006), the increase of survival time during the SEs may most likely be related to the fertilization of coastal waters during upwelling events and the consequent enhancement of primary and secondary production in the Iberian region (Tenore et al. 1995, Guisande et al. 2001). In line with this rationale, the positive correlation recorded between CL and survival time may also be supported by the upwelling index. As time to metamorphosis did not differ significantly among events, it is legitimate to assume that megalopae from all these events may have made a similar energetic investment to metamorphose when stocked in the laboratory under identical conditions. In this way, the differences recorded in survival time may only be explained through the differential levels of energy accumulated by early stage megalopae (and previous zoal stages) before re-invading their adult habitat. Marine invertebrate larvae are specialized in morphogenesis and growth, conditioning their feeding behaviour to maximize their performance (Calado & Leal 2015). Therefore, during planktonic life, larvae are well adapted to the patchy nature of the plankton, exploiting in a very efficient way even brief or intermittent periods of food availability (Harms et al. 1994, Giménez & Anger 2005, Calado & Leal 2015). Under favourable feeding conditions, developing larvae may easily accumulate and store high quality energetic reserves (Wang et al. 2014). Such energetic reserves will fuel subsequent periods of their life, namely energy-demanding processes associated with metamorphosis and early post-settlement life. Studies performed in other invertebrates, which also combined field and labora-

tory experiments, found a positive relationship between quality of settling larvae (e.g. larval nutritional conditions, larval size) and post-settlement performance (Phillips 2002, 2004, Jarrett 2003, Marshall & Keough 2004).

The second null hypothesis tested in this study was also rejected, as, under standard laboratory conditions, the performance of early juvenile crab instars significantly shifted with the SE of the megalopa and with upwelling conditions experienced during planktonic larval development. Juvenile crab traits surveyed during the present study (CW C1 and C5; and WW C5) kept a similar trend to that already described above for the megalopae, thus supporting the existence of carry-over effects from pelagic larval life to early benthic juvenile stages—as already highlighted by Pechenik (2006) ‘metamorphosis is not a new beginning’. Settlers originating from SE-1 and SE-2 presented a lower CW (at C1 and C5), as well as WW (C5), than those from SE-3 and SE-4, despite being stocked under identical culture conditions (e.g. food supplied ad libitum, constant temperature and salinity within optimal ranges for the species). This record evidences that early benthic life even under optimal conditions is not sufficient to erase the fingerprinting of larval life in the pelagic environment.

The analysis performed using GAMs showed that most of the deviance explained for megalopae size (i.e. CL) and juvenile crabs' performance (i.e. CW of C1 and C5 and WW of C5) were obtained with an upwelling index averaging between 40 to 50 d before the settlement event, with the exception of survival time where the estimated upwelling index average was 30 d. The larval development of *C. maenas*, as in most marine invertebrates, is mainly controlled by temperature and food (Dawirs 1984, 1985, Calado & Leal 2015). The average temperature of the water in the months that preceded our sampling period commonly ranges between 12 and 15°C (Domingues et al. 2011, Álvarez et al. 2013). Thus, according to Dawirs (1985), larval development of *C. maenas* is expected to range from 40 to 60 d from hatching to metamorphosis. In this way, it can be assumed that according to GAMs larval and juvenile traits recorded in the present study were determined by the nutritional conditions experienced by early larval stages (the zoea), as well as those experienced by the megalopae before reaching their PRS and becoming competent. The role played by the nutritional conditions experienced by megalopae at settlement will likely play a secondary role on their life post-metamorphosis. The positive influence of upwelling con-

ditions on survival time (57.3% of deviance explained) suggests a relevant role of nutrient enrichment of the coastal waters during larval development. The low deviance explained (26.2%) in the CL analysis might be related to other factors which also condition larval phenotypes (e.g. physiological stress and maternal effects) (Marshall et al. 2008). Therefore, GAM results suggest an important role of larval history in juvenile performance, which can be explained by the occurrence of carry-over effects (Pechenik 2006). Calado et al. (2005) and Calado (2008) showed that starving newly hatched larvae of a caridean shrimp and forcing them to catabolize their reserves to moult to the second larval stage (Zoea II) only promoted detectable effects 20 to 40 d post-hatching (e.g. survival, larval stage duration and time of metamorphosis), and that these were carried over to early benthic life. Juvenile shrimp originating from larvae starved at hatching always displayed a poorer growth performance than conspecifics originating from larvae that never experienced suboptimal feeding. In this way, environmental conditions prevailing during planktonic development (e.g. temperature, available nutrients) may condition larval performance (e.g. nutritional status, size) and these effects can persist during early benthic life.

Available data from long-term sampling of settlement events of marine invertebrates with bi-phasic life cycles have revealed the existence of a considerable variability in larval supply (Domingues et al. 2011, Peteiro et al. 2011, Pfaff et al. 2015), as well as in the quality of settling larvae (Phillips 2002, Jarrett 2003, Giménez 2010, Cruz et al. 2010, Abdul Wahab et al. 2014). Our study showed that, even within a reduced time frame (i.e. supply events with the highest number of megalopae being recorded along the settlement season), the larval stages being supplied to undergo metamorphosis in the habitat occupied by their adult form displayed a variable post-metamorphosis performance. This natural variability appears to be associated with phenotypic plasticity, with GAM analysis exposing the relationship between larval and juvenile performance and the upwelling index prevailing during larval pelagic life. As larval survival and development are shaped by the quantity and quality of available food (Boidron-Métairon 1995), pelagic conditions experienced during larval life can determine the growth performance of juvenile specimens and their ability to recruit to the adult population (e.g. by reaching sexual maturity). Maternal investment is also known to play a key role in the success of early larval life (Marshall &

Keough 2006, Marshall et al. 2008). It is now clear that researchers may only perceive the mechanisms ruling the population dynamics of marine invertebrates with bi-phasic life cycles by considering the driving force exerted by trait-mediated effects. It is therefore important to increase the number of studies that monitor not only the timing and quantitative supply of competent larvae, but also their quality in terms of survival and early benthic performance. Clarifying the origin of trait-mediated effects, and being able to dissociate between maternal effects and environmental conditions as a source of natural variability during early benthic life, will be a challenge for future studies. These topics are paramount to develop a unifying framework to better understand the population biology of marine invertebrates with bi-phasic life cycles.

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