

Persistent and context-dependent effects of the larval feeding environment on post-metamorphic performance through the adult stage

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2 3 4	PERSISTENT AND CONTEXT-DEPENDENT EFFECTS OF THE LARVAL FEEDING ENVIRONMENT ON POST-METAMORPHIC PERFORMANCE THROUGH TO THE ADULT STAGE
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ABSTRACT

One of the central issues in ecology is the identification of processes affecting the 28 population structure and dynamics of species with complex life cycles. In such species, 29 variation in both the number of larvae that enter a population and their phenotype are 30 31 important drivers of survival and growth after metamorphosis. Larval experience can have strong effects on key post-metamorphic traits but the temporal scale of such "trait-mediated 32 effects" may be short and their magnitude may depend on the environment experienced after 33 metamorphosis. We used an intertidal barnacle to study the long-term consequences of trait-34 35 mediated effects under different post-metamorphic conditions by manipulating larval food concentration and monitoring patterns of survival and growth in juveniles at two intertidal 36 levels over a 5 month period. In two replicated experiments higher food levels resulted in 37 increased body size, mass and reserves (measured from elemental composition) in the settling 38 39 larval stage and increased body size of newly metamorphosed juveniles. In Experiment 1 high food concentration reduced juvenile mortality at low intertidal levels while on the upper 40 41 intertidal, mortality was high for all larval food concentrations. By contrast, in Experiment 2 low larval food concentration decreased juvenile survival at both shore levels. When present, 42 43 effects were established early (weeks 1 or 2) and persisted for over 10 weeks in Experiment 1 and 22 weeks in Experiment 2. Interactive effects of the larval and juvenile environments can 44 have important implications for population size: trait-mediated effects may persist for long 45 periods contributing to explain patterns of adult abundance. 46

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INTRODUCTION

For species with complex life cycles, a better understanding of processes affecting the 57 structure and dynamics of populations and communities is achieved if studies consider both 58 pre- and post-metamorphic stages (Thorson 1950, Grosberg & Levitan 1992, Caley et al. 59 1996, Jenkins 2005, Allen & Marshall 2010, Marshall & Morgan 2011). In marine 60 invertebrates, the number of individuals successfully settling and metamorphosing, as well as 61 the patterns of recruitment, can vary enormously over a number of spatial and temporal scales 62 (e.g. Jenkins et al. 2000, Navarrete et al. 2002, Broitman et al. 2008); in barnacles such 63 64 variation has been attributed to patterns of predation (Gaines & Roughgarden 1987), behaviour (Jenkins 2005) and transport by currents (Roughgarden et al. 1988). While for 65 66 many decades benthic ecologists focused on post-settlement processes to explain patterns of community structure, the discovery of the role of the pre-settlement processes changed the 67 68 views about the organization of marine communities, which now recognises the balance 69 between the role of pre- and post-metamorphic processes operating on cohort dynamics 70 (Connell 1985, Gaines & Roughgarden 1985, Menge 2000, Jenkins et al. 2008). At a wider scale the consideration of pre-settlement processes on connectivity, has contributed to the 71 72 development of the field of marine metapopulation dynamics (Armsworth 2002, Shima & 73 Swearer 2009). In addition, a growing body of work has also shown that the environmental conditions experienced by larval stages can affect the phenotype and eventually the 74 performance and survival after metamorphosis (Prout & McChesney 1985, Giménez 2004, 75 76 Pechenik 2006, Aguila et al. 2013). These effects called "trait-mediated effects" (Giménez 2004, Kerby et al. 2012), are part of a wider type of plastic response where traits of 77 organisms are altered in response to biotic and abiotic pressures (Miner et al. 2005); these 78 effects are widespread among organisms, and have important consequences for the 79 organization of communities (Schmitz et al. 2003, Werner & Peacor 2003, Ohgushi et al. 80 81 2012).

The most widely studied type of trait-mediated effect is perhaps that operating top-down, where morphological or behavioural traits of a consumer are modified by the presence of predators (Kerby et al. 2012); in tri-trophic food chains, the response of the consumer to predator cues can modify the abundance of the producer. There are also bottom-up effects where, for instance, food availability or the physical environment experienced by early (e.g. larval) stages affect physiological or morphological traits of advanced stages, and subsequently their chances of survival, and recruitment (Giménez 2004, Pechenik 2006).

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89 Here, the emphasis is on the consequences of modified traits as they propagate through the life cycle. We know that the larval environment can have a profound influence on individual 90 size and available reserves at the time of metamorphosis. A range of studies have clearly 91 demonstrated that over the first days of post-metamorphic life the larval environment can 92 determine metamorphic success (e.g. Tremblay et al. 2007), survival (Pechenik et al. 1993) 93 and the ability to tolerate food limitation (Thiyagarajan et al. 2003 a, b) or physical stress 94 95 (Phillips 2002). However, we still do not clearly understand the long term consequences of trait-mediated effects propagating through the life cycle, for instance if effects of early (e.g. 96 97 larval) experience will reach beyond a few days after metamorphosis. Strong effects, i.e. those that can influence population dynamics, should have long-term consequences on 98 fecundity or on the number of individuals reaching reproductive maturity. The strength of 99 such effects may be restricted to species with a short post-metamorphic phase. In species with 100 a short juvenile phase (<4 weeks) the larval environment can affect fecundity (Prout 101 &McChesney 1985, Wendt 1998); extreme cases are the holometabolous insects where 102 feeding larvae eclose into an adult stage whose energy reserves largely depend on larval 103 104 history (Aguila et al. 2013).

105 In addition, environmental conditions experienced after metamorphosis modify the strength of a trait-mediated effect leading to context-dependent effects. For instance, 106 environmental stochasticity, experienced at advanced stages, may also limit the strength of 107 trait-mediated effects because it may blur the relationship between the larval environment, 108 post-metamorphic phenotype and survival. In species with long post-metamorphic phases 109 (months to years), laboratory studies where environmental conditions are kept constant, show 110 that effects of larval experience on phenotype are still found ca. three months after 111 metamorphosis (Giménez et al. 2004, Giménez 2010). However, relationships between early 112 and late phenotypes are sometimes weak in the wild (Lindholm et al. 2006, Auer et al. 2010), 113 114 where conditions in the post-metamorphic environment can re-shape phenotypes (and modify fitness) or produce immediate effects on mortality, irrespective of the phenotype. 115

116 Context-dependent effects are important even in the absence of environmental 117 stochasticity but the lack of research (Allen & Marshall 2013) still precludes the formulation 118 of specific predictions about which environmental contexts enable trait mediated effects to 119 influence recruitment. While some studies have shown trait-mediated effects when post-120 metamorphic conditions are harsh (Spight 1976, McGinley et al. 1987, Hutchings 1991, 121 Tamate & Maekawa 2000, Phillips 2002, Allen & Marshall 2013), the opposite pattern has 122 also been reported (Moran & Emlet 2001). Most likely, contradictory results reflect different type of stressors (Moran 1999) or non-linear responses to a stressor (Allen et al. 2008): i.e. 123 the fact that under extremely harsh conditions all organisms die irrespective of traits, while in 124 benign conditions trait-mediated advantages are too small. In the first case, some specific 125 stressors may select for particular body sizes, while other stressors may not (Moran 1999). In 126 the latter case, trait-mediated effects may arise if environmental conditions are intermediate 127 between the extremes described above (Allen et al. 2008). More complex responses that have 128 129 been found in field studies evaluating larval responses to egg size and thermal conditions, i.e. 130 across another life history boundary, suggest that complex patterns are possible. For example in the frog Bombina orientalis larvae hatching from large eggs perform better at low 131 temperatures or under low variability in temperature but the patterns reverse at high 132 temperatures (Kaplan 1992, Kaplan & Phillips 2006). 133

134 In marine benthic invertebrates, observations suggest mortality is generally high throughout a range of taxa over the period following metamorphosis (Gosselin & Qian 1997, 135 136 Hunt & Scheibling 1997, Underwood & Keough 2001, Gosselin & Jones 2010). However, mortality at advanced juvenile stages can also be high if intraspecific competition increases 137 138 as individuals use more resources (Jenkins et al. 2008, Giménez & Jenkins 2013), and modelling output indicates that juvenile/adult survival is critical to local dynamics (Svensson 139 et al. 2004). These results suggest that a longer term perspective of trait mediated effects, 140 integrating across life stages, is required. 141

In this paper we address the questions of the long term consequences of trait mediated 142 effects under different post-metamorphic contexts. We studied the effect of larval food 143 environment on larval quality and subsequent long term post-metamorphic survival and 144 growth of an intertidal acorn barnacle in two habitats characterised by different levels of 145 environmental stress over a period of 22-25 weeks. The study addressed the following 146 147 questions: (1) What is the relationship between the larval environment and the phenotype 148 before and after metamorphosis? (2) Do we see trait-mediated effects? (3) Do these traitmediated effects propagate through time or alternatively does stochastic variation override 149 150 the signal? (4) If present, do trait-mediated effects depend on the environmental context (tidal elevation)? 151

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METHODS

155 <u>The model species</u>

Intertidal barnacles are a useful model system to address trait-mediated effects on 156 population dynamics. They develop through a series of pelagic larval feeding stages, the 157 nauplius, followed by a non-feeding larval stage, the cyprid, which settles and 158 159 metamorphoses. Food conditions experienced by nauplius stages determine the amount of 160 reserves available to the cyprids to search for an appropriate settlement site and undergo metamorphosis (West & Costlow 1987, Hentschel & Emlet 2000, Thiyagarajan et al. 2003 a, 161 b). Metamorphosis requires a considerable amount of total available reserves (e.g. 30% in 162 Semibalanus balanoides: Lucas et al. 1979) and feeding does not start until 2-5 days after 163 metamorphosis (Rainbow & Walker 1977). Hence it is not surprising that both laboratory 164 (Thiyagarajan et al. 2003a) and short term field studies (Jarrett 2003, Tremblay et al. 2007) 165 have found that metamorphic success and early post-metamorphic survival are influenced by 166 the larval food environment and positively correlate with the amount of cyprid reserves 167 (Jarrett & Pechenik 1997, Miron et al. 1999). However, the long term effects of the larval 168 environment and how this interacts with levels of post larval environmental stress are not 169 known. 170

171 Austrominius modestus, a non-native species originally from Australasia, was first recorded in the UK in 1945 (Crisp 1958) and since then it has spread rapidly throughout the 172 173 European continent (Harms 1986). The duration of larval development, through six naupliar stages followed by the cyprid, depends on temperature: in the Irish Sea, larvae are expected 174 175 to take ca. 15 days (Harms 1999) to reach the first juvenile stage. In the study area, larval 176 development and settlement takes place mainly during the summer through to early autumn. 177 Juveniles feed on plankton at high tide, grow rapidly and are able to breed within 12 weeks 178 (Crisp & Davies 1955).

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Laboratory and field procedures

Adult *Austrominius modestus* were collected from the mid intertidal zone of Menai Bridge (Isle of Anglesey, UK), and maintained in the laboratory in seawater. In two separate experiments, in September and October 2011, larval release was stimulated by detaching the adults from the rock. For each experiment freshly hatched larvae, from ca. 100 adults, were pooled and then divided among 18 51 vessels. Nauplii were mass-reared at an initial density

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185 of 0.8-1.0 individual per ml at three different food concentrations (6 replicate vessels per food treatment) using the diatom Skeletonema costatum as food (Harms 1987). Larvae were reared 186 following Harms (1987) at low ($1x10^5$ cells ml⁻¹), medium ($2x10^5$ cells ml⁻¹) and high ($3x10^5$ 187 cells ml⁻¹) food concentrations at 16°C under gentle aeration. These concentrations produced 188 low larval mortalities in preliminary experiments. Water and food were changed every 189 second day and dead larvae discarded. Towards the end of each experiment, water was 190 changed daily and cultures were inspected for cyprids. When cyprids amounted to 50-80% of 191 larvae present (in most cases ca. 24-48 h from when the first cyprids were observed) the 192 193 contents of each culture vessel were transferred to a separate settlement vessel made of PVC, each containing 6 natural slate tiles of 3x3 cm each (i.e. there was a settlement vessel 194 associated to each replicate culture vessel). After 48 hours, tiles with settlers were out-planted 195 to the field and remaining swimming cyprids discarded, to avoid confounding food treatment 196 effects with effects of delayed metamorphosis. Development time to reach the cyprid stage 197 varied slightly among food treatments such that transfer to the settlement vessel and 198 subsequent settlement was delayed by one and two days in intermediate and low food 199 concentrations respectively compared to high food. Rather than maintain settlers from 200 different food treatments under lab conditions for differing periods, out-planting was 201 202 performed at the end of the settlement period and hence out-plant dates differed by a maximum of 2 days among food treatments. Most tiles (90%) had densities below 5 ind*cm⁻ 203 ²; the maximum density of settlers per tiles was 93 (~10 ind*cm⁻²); density did not vary in 204 any consistent way among food treatments. Observations showed that settled individuals 205 206 were unlikely to compete for space or resources since there was enough free space between settlers until the end of the experiment. Therefore food effects were not confounded with 207 208 density effects.

Tiles were out-planted (Experiment 1: 21-23 Sept 2011; Experiment 2: 17-19 Oct 2011) on a rocky intertidal outcrop under the suspension bridge in the Menai Strait (ca. 800m from the laboratory) at two tidal levels, 4.8 m and 3.0m above Chart Datum, corresponding to the upper and lower distribution of *Austrominius modestus*. Three PVC frames were used at each tidal level and tiles (2 to 3 from each vessel) were attached using a 5mm pre-drilled hole through the tile centre at random across these frames. In total between 100 and 400 individuals were out-planted per treatment combination.

All tiles were photographed, to determine survival and growth rates, before out-planting and then at bi-weekly (weeks 2-10) intervals, and at the end of the experiments in March

2012 (Experiment 1: 25 weeks and Experiment 2: 22 weeks). In addition, in Experiment 2, 218 tiles were also sampled one week after out-planting. During the first two weeks, tiles were 219 photographed under a dissecting microscope (Leica Microscope MZ 6) by transporting tiles, 220 attached to the PVC frames, to the laboratory during low tide, and returning before the 221 222 incoming tide. Subsequently, barnacle sizes were large enough to allow appropriate estimations of body size through in situ photography (Pentax Optio W60 camera mounted on 223 a PVC frame). Digital images were processed using Image J software; all surviving 224 individuals were counted and the basal and operculum length measured in 5 individuals from 225 226 each replicate settlement vessel. Body size measurements ended when less than 5 individuals per replicate vessel remained on the tiles (week 10 for Experiment 1 and week 22 for 227 Experiment 2). 228

229 Body size, dry mass and elemental composition of swimming cyprids

In both experiments cyprid body size was determined by measuring 20 cyprids per 230 replicate vessel under the microscope; cyprids were collected as swimming individuals within 231 the first 48h of the first cyprids being observed. In Experiment 2, dry mass and elemental 232 composition were also determined by sampling 100 swimming cyprids from each replicate 233 vessel. Sample processing followed Anger & Harms (1990): 100 individual cyprids were 234 pipetted out of each replicate vessel, quickly rinsed in distilled water, blotted dry with filter 235 paper, placed in aluminium cartridges and frozen at -20°C for later analysis; 20 randomly 236 chosen individuals per sample were measured under the microscope before being placed in 237 the cartridges. Samples were freeze-dried (Edwards Supermodulyo 12 k freeze-drier) and 238 239 weighed using a microbalance (Mettler Toledo, precision = $1 \mu g$). Elemental composition (Carbon and Nitrogen content) was determined using a CHNS-O Analyser (Thermo Electron 240 Flash EA 1112 Series). 241

242 <u>Statistical analysis</u>

We used each culture vessel, and corresponding settlement vessel, as a replicate unit, such that all tiles originating from each vessel were considered as one replicate. A minimum of five vessels from each food treatment produced suitable tiles. Statistical tests were run for each experiment separately. We first tested if food concentration affected cyprid body size, dry mass or elemental carbon and nitrogen content. For body size we obtained data from individual cyprids: therefore, a nested ANOVA was used with food concentration as a fixed factor and culture vessel nested within food concentration (replicate unit = individual larvae sampled from within each vessel). A one-way ANOVA was used for dry mass and elemental
composition where one sample per vessel (made up of 100 cyprids) was obtained. After
significant differences in ANOVA, differences among treatments were tested here and in
subsequent analyses using SNK posthoc tests.

We tested if the body size of metamorphs (basal and operculum length) varied between intertidal level and larval food using a two-way ANOVA. Our analyses confirmed that body size did not differ among intertidal levels at the time of out-planting (see results).

The effects of larval food concentration, intertidal level and time on survival were tested through a 3-way repeated measures ANOVA using each of the settlement vessels as our replicate unit (i.e. values from tiles within each settlement vessel were combined). Variances were homogeneous (Cochran's test) and residuals did not show any serious deviations from the normal distribution.

Since the highest mortality rates were observed during the first 2 weeks (see results), we 262 also tested for potential effects of initial densities of post-metamorphs on the proportion of 263 264 barnacles surviving the first 2 weeks in the intertidal. This test considered interactions of 265 initial barnacle numbers, larval food and intertidal level and was made using tiles (instead of vessels) as this was the natural replicate unit to express densities. Tests were run using 266 267 general least square (gls function in nlme package: Pinheiro 2015) using the VarPower constructor function (variance depended on barnacle density). Pearson residuals showed 268 269 homogeneity and did not show serious deviations from normal.

The effects of larval food concentration, intertidal level and time on body size of metamorphs (basal and operculum length) were tested using Generalized Linear Modelling (GzLM) with Gamma distribution and logarithmic link function. ANOVA was not used because variances were heterogeneous and did not follow a normal distribution even after data transformation.

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RESULTS

278 Effect of food concentration levels on traits of swimming cyprids and metamorphs

For both Experiments 1 and 2, larval food concentration significantly affected cyprid body length (Table 1), with low food concentration resulting in a 4 to 7% reduction in size compared with those from the high food level. Intermediate food concentrations resulted in cyprid lengths equivalent to the high food treatment in Experiment 1 but an intermediate size in Experiment 2 (Fig. 1).

284 Dry mass (DW) and elemental composition were only measured in Experiment 2. At low food concentrations cyprid DW was 41% lower than at intermediate and high food levels 285 (Table 2: significant food effect, Fig. 2a). Significantly lower carbon (C) and nitrogen (N) 286 287 content per individual were also found under low food levels (data not shown). The amount 288 of carbon per individual cyprid, for example, was 47% lower at low food concentrations 289 compared to high. Levels of C and N relative to DW also responded to food treatments: % C was significantly greater in the high food treatment with 9.5% and 7% lower values in low 290 291 and intermediate food treatments respectively (Table 2, Fig 2b). In contrast to all other patterns % N was highest in the low food treatment, and significantly lower in the mid and 292 293 high treatments (11-13% lower than in high food treatment, Fig. 2c). The strong food effects on C compared to N led to significant differences in C:N ratio among all treatments (high to 294 295 mid to low food); in larvae reared under low food concentrations this ratio was 21% lower 296 than in those reared under the highest food concentration (Fig. 2d).

297 At the time of out-planting, body size (basal and operculum length) of metamorphs (within 24hs. of metamorphosis) varied among food concentrations but not between intertidal levels 298 (Table 3, Fig. 3) showing that individuals of different sizes were effectively allocated 299 randomly among intertidal levels. In both experiments, the highest food concentration 300 resulted in the largest basal length after metamorphosis (Fig. 3 a-b). Low food concentration 301 resulted in metamorphs that were 15% (Exp-1) and 8% smaller (Exp-2) in basal length than 302 those from high food concentration. The operculum length was largest for individuals 303 304 metamorphosed from larvae reared under high food concentrations in Experiment 1 (Fig. 3c), 305 but similar sizes were found between individuals reared under high and medium food concentrations in Experiment 2 (Fig. 3d). 306

It is interesting to note in Experiment 1 the way in which cyprid size responded differently to food treatments than metamorph size (Fig 1a vs. Fig 3a). The medium food concentration produced cyprids equivalent in body length to those at high food concentrations. However, this size advantage over the low food treatment was not maintained in metamorphs, where the medium food concentration clearly produced metamorphs equivalent to those from low food
with a basal and opercular length on average 16% and 13% respectively, smaller than in the
high food treatment.

314 <u>Post-metamorphic survival</u>

In both experiments the percentage of out-planted barnacles surviving to a specific week 315 316 (cumulative survival) decreased strongly during the first 2 weeks and then remained steady over the study period (Fig. 4). In Experiment 1 (Fig. 4 a-b), the effect of larval food 317 environment on cumulative survival depended on intertidal level (Table 4, significant 2-way 318 interactions). Significant effects of larval food concentration were restricted to the lower 319 intertidal: high larval food concentrations resulted in the highest survival; the differences 320 321 between low and intermediate food concentrations were not significant. This effect of food on survival on the lower intertidal was established between the time of out-planting and week 2 322 (06/10/11). On average 65% of metamorphs originated from the high food level survived the 323 first two weeks after out-planting; only 37-46% of those from the intermediate and low food 324 level survived that period (Fig. 4a, SNK posthoc tests). By contrast, in the upper intertidal 325 survival was low irrespective of the larval food treatment (on average 25% of the out-planted 326 327 metamorphs, Fig. 4b).

328 Further examination of Experiment 1 shows that the effect of food observed in the low intertidal at week 2, remained (except in week 6) until week 10 (Fig. 4a, SNK posthoc test) 329 330 owing to a bi-weekly survival (percentage surviving any two-week period) which was consistently high (>70%) irrespective of food treatment. By week 10 the cumulative survival 331 332 was on average 37% in juveniles metamorphosed from larvae reared at high food 333 concentrations, significantly higher than those at intermediate and low food concentrations 334 which showed an average survival of 15 and 24%, respectively (Fig. 4a). At week 25 335 (16/03/12) the effect of larval food concentration on cumulative survival was not significant, but the trend was still present (Fig 4a). The loss of significance was most likely due to loss of 336 power in the test since very few individuals (<5 per tile), remained alive at that time. In 337 summary, in Experiment 1, the effect of larval food on barnacle density, found in the low 338 intertidal level, was established in the first two weeks; these differences in barnacle density 339 due to the effect of larval food were maintained from week 2 until week 10. 340

In Experiment 2 there was a significant main effect of larval food concentration which was consistent across both intertidal levels (Table 4); cumulative survival was lowest in

metamorphs which originated from the lowest food concentrations while those from 343 intermediate and high food concentration showed similar levels of survival (Fig. 4 c-d, SNK 344 posthoc test). In this experiment the effect of larval food conditions on survival was apparent 345 one week (24/10/11) after settlement and these differences remained over the whole 22 week 346 study period. The percentage survival after two weeks was on average 34% for the 347 metamorphs from the low food level and 52 % for those from high and intermediate food 348 levels. After the second week, bi-weekly survival was high (on average >80%) irrespective of 349 food treatment. Thus, the differences in barnacle density related to larval food environment, 350 351 were established during the first two weeks and remained for 22 weeks of the study period.

Examination of survival as a function of density revealed inconsistent patterns. For Experiment 1, initial density (D) and proportion of survivors (S) after 2 weeks were weakly but negatively correlated (S = 0.48 - 0.0022 D, p = 0.016) irrespective of the food and intertidal level. For Experiment 2, the correlation of initial density and survival depended on the intertidal level: for the high level the correlation was positive (S = 0.54 + 0.0025 D, p = 0.04), and for the low level, the correlation was not significant; again.

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359 <u>Post-metamorphic growth</u>

Overall, barnacles grew from ca. 0.55 to 4-5 mm in basal length (Fig. 5) and from 0.39 to 360 1.5-2.5 mm in operculum length (data not shown). In Experiment 1, the effect of food 361 concentration was found only in the lower intertidal (intertidal level x food interaction: Table 362 5): high food concentration led to significantly larger body size on the lower intertidal, (basal 363 length, Fig. 5a) and operculum length (not shown) than the intermediate and lower food 364 concentrations (SNK posthoc test). These differences were established at the time of out-365 366 planting (see Fig. 3 for details) and appeared to increase with time (Fig.5a). Initially the basal length of metamorphs from the low and intermediate food treatments were on average 0.12 367 mm smaller than those from the high food treatment (0.55 vs 0.67 mm); this difference 368 increased to 1mm after 10 weeks. By contrast, proportional differences varied little between 369 the time of out-planting (17%) and after 10 weeks (19%). There was no effect of food 370 treatment on growth in Experiment 2. 371

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DISCUSSION

374 In species with complex life cycles, spatial and temporal variation in the timing of metamorphosis can be important in determining the structure and dynamics of populations 375 and communities (Gaines & Roughgarden 1985, Caley et al. 1996, Connolly et al. 2001, 376 Jenkins et al. 2008) and metapopulation persistence (Armsworth 2002). However, recent 377 work shows that variations in traits (e.g. body size, nutritional reserves), at or after 378 metamorphosis, also affect subsequent survival or reproduction (Pechenik 2006) and can 379 translate into effects on recruitment (Giménez 2004) and reproductive potential for a 380 population (Burgess & Marshall 2011). Such trait-mediated effects may be strong in species 381 382 with a short post-metamorphic phase. However, it is not straightforward to expect similar effects for species with longer post-metamorphic life (Pechenik et al. 1998) because post-383 metamorphic conditions (i.e. stochasticity, biotic interactions, stress, disturbance or density-384 dependent effects) may prevail over any effect produced by the pre-metamorphic 385 environment. Using an intertidal barnacle as a model we found: (1) that effects of the larval 386 environment on performance, when present, had long term consequences, affecting the 387 abundance and size of individuals reaching reproductive maturity; (2) context-dependent 388 389 effects of the larval environment on performance, mediated by changes of larval and post-390 metamorphic traits. In addition, we found: (3) variable responses among experiments that 391 may reflect variations in the environmental context or other sources (e.g. genotype x environment interactions). Long-term but variable effects (context-dependent or not) add to 392 393 the complex ways in which trait-mediated effects can affect natural communities (Werner & Peacor 2003, Ohgushi et al. 2012). 394

395 The persistence of trait-mediated effects is critical in demonstrating that larval traits can have a strong influence on population level processes. We showed persistence of effects from 396 397 the time of settlement in autumn until the spring (an age at which A. modestus can be reproductively mature: Crisp & Davies 1955). Most studies demonstrating effects of larval 398 399 history on performance, focus on the first 2-3 weeks after metamorphosis (Pechenik et al. 1993, Phillips 2002, Thiyagarajan et al. 2003 a, b). Temporal persistence of larval effects is 400 401 not widely known for marine invertebrates (but see Allen et al. 2008) and we are not aware of 402 any field study tracking cohorts of invertebrates for several months after manipulating the larval environment. Previous studies, in species with short maturation times, have shown 403 404 important effects of the larval environment on adult cohorts (e.g. Prout & McChesney 1985; Wendt 1998) or effects of the natal habitat on population dynamics over several generations 405 (Van Allen & Rudolf 2013). Our results extend those carried out with short post metamorphic 406

407 phases and point to the potentially widespread effect of the larval environment on 408 recruitment. There is now an important body of work that highlights the contributory role of 409 oceanographic conditions in determining the recruitment of individuals to adult stages, 410 through effects on larval settlement (Connolly et al 2001). In addition, variations in 411 oceanographic conditions leading to, for example, changes in food availability, may also 412 contribute to changes in recruitment through modifications of traits at or after 413 metamorphosis.

414 During our study, it was striking to observe that patterns in survival, once established, persisted over a long period irrespective of biotic and abiotic processes operating after 415 416 metamorphosis. These patterns were established during the first two weeks when mortality in 417 invertebrate juveniles is known to be particularly high (Gosselin & Qian 1997, Hunt & Scheibling 1997, Underwood & Keough 2001, Gosselin & Jones 2010); on average, 58% of 418 419 the out-planted juveniles were lost during that period. In the case of barnacles, the level of reserves at metamorphosis is critical since for the following few days they cannot feed 420 421 (Rainbow & Walker 1977); therefore, it is likely that there is a critical window where effects of larval experience on post-metamorphic survival are highest. 422

A potential process leading to high early mortality is density-dependence, for instance 423 competition. In our case however, density-dependence did not seem to be an overall 424 explanation for the high mortality observed in both experiments. Significant negative 425 correlations between densities and survival were found only in Experiment 1. Competition 426 was unlikely because metamorphs were distributed randomly over the plates, at low densities 427 (max. density = $10 \text{ ind}^{*}\text{cm}^{-2}$), at such distances that they would not have opportunity to 428 engage in competition. A previous study on barnacle density-dependence carried out also on 429 Welsh intertidal shores, albeit on *Semibalanus balanoides* (Jenkins et al. 2008), suggest that 430 such process requires higher densities (above 20 ind*cm⁻²) for 1 month old juveniles that are 431 432 larger and occupy more space than 2 week old Autrominius modestus.

Patterns established early in the benthic phase persisted because actual mortality rates did not vary further among food treatments (only 16% were lost in any subsequent 2 week period) leading to the so called type III trajectory (Caley 1998). We can only speculate about the reasons behind the maintenance of the patterns. The timing of our experiments meant that surviving juveniles developed through autumn-winter conditions when low temperatures may reduce the strength of biotic interactions or metabolic requirements. Perhaps the timing of settlement in relation to the seasonality in the environment is an important factor determining
the extent of trait-mediated effects. In addition, a reduction in sensitivity to environmental
conditions through ontogeny could also be important (McCormick & Hoey 2004).

442 It is important to understand the physiological mechanisms leading to trait-mediated effects to progress toward a predictive theoretical framework. In this particular case, the 443 mechanisms leading to trait-mediated effects may involve processes occurring before, during 444 and after metamorphosis. First, low larval food concentration resulted in a reduction in cyprid 445 size, % C content and body mass (DW) as well as a reduced C:N ratio, effects which are 446 447 consistent with findings for other barnacles (Thiyagarajan et al. 2002 b, Emlet & Sadro 2006); most of the changes in C content may result from reductions in the proportion of total 448 449 lipids or triacylglycerols, which have been linked with variations in growth and survival of early barnacle stages (Thiyagarajan et al. 2002 a, b; Tremblay et al. 2007). Second, important 450 451 changes appeared to occur during metamorphosis because differences in body size among larval food treatments were not fully equivalent between pre-metamorphic (cyprid) and post-452 453 metamorphic juvenile stages. For example, in Experiment 1 intermediate levels of food produced larger cyprids which were equivalent in size to the high food treatments, but 454 metamorphs that were smaller and equivalent to individuals raised on low food concentration; 455 a similar mis-match occurred in Experiment 2. In addition, examination of standardised 456 average values of cyprid and metamorph size and early survival show clearly that survival 457 was fully linked to metamorph, but not cyprid size (Supplementary figure). Overall, these 458 findings emphasise the importance of the interaction between physiological processes 459 determining larval traits and the process of metamorphosis in establishing early post-460 metamorphic traits, which appeared to underpin the patterns of survival. 461

Another important result was the context-dependent nature of the trait-mediated effects. In 462 Experiment 1, the effects of larval environment on survival were only evident in the lower 463 intertidal; in the upper intertidal, where conditions are expected to be more stressful (longer 464 465 daily periods of desiccation, extreme temperatures and lower food supply) survival was strongly depressed, irrespective of food quality. Most related studies argue that the benefits of 466 better quality larval phenotype will be expressed in poorer quality environments (e.g. Spight 467 1976, McGinley et al. 1987, Hutchings 1991, Tamate & Maekawa 2000, Phillips 2002, Allen 468 469 & Marshall 2013) yet our work did not show this. Observations similar to our own have been made by Moran & Emlet (2001) who showed that hatching size of the gastropod Nucella 470 471 ostrina positively affected early survival in a benign shaded habitat but not in a stressful sun472 exposed environment. It is likely that under the conditions tested in our first experiment, the 473 feeding/desiccation conditions were too harsh in the upper intertidal, but not in the lower 474 intertidal. The limited number of studies and contradictory results still precludes making any 475 generalization about how variations in traits of metamorphs affect recruitment along the 476 intertidal gradient.

The still limited capacity for generalization is further shown by our results from the 477 second experiment, where trait-mediated effects were found at both levels; this is relevant as 478 479 a warning for interpreting results of studies lacking any level of repetition. We can only 480 speculate that either environmental variability or variability among cohorts of settling larvae 481 may drive trait-mediated effects. Evidence in favour of an environmental effect, in particular 482 thermal stress, comes from naturally occurring differences in temperature experienced by juveniles out-planted in the different experiments. Temperature records (Hilbre Island 483 484 meteorological station) show that the average air temperature during the first two weeks after the out-planting in September (17.8°C,) was five degrees higher than in that experienced by 485 486 barnacles out-planted in October (13.2°C); during the same period, daily temperature maxima (September: 25°C; October: < 20°C) coincided with midday/early afternoon low water 487 periods. These data, combined with the laboratory observations of Foster (1971) of 50% 488 mortality rates of A. modestus recruits at 20°C, suggest that high intertidal level out-plants in 489 Experiment 1, where larval food treatment effects were not observed, would have been 490 exposed to potentially much higher levels of emersion stress than those in Experiment 2, 491 where trait-mediated effects were clear. An alternative view of our results is that the different 492 493 outcomes of the two experiments may reflect variations in larval phenotypes among cohorts. Evidence in favour of this hypothesis is that the effect of the larval food concentration on 494 495 basal and operculum diameter was weaker in the cohort out-planted in October than that out-496 planted in September; hence, that cohort would have been better suited to tolerate the 497 conditions existing in the upper intertidal level. Variations in phenotypes may reflect genetic variability or maternal effects on egg sizes and embryonic development. Variations in egg 498 499 size within populations are important in intertidal barnacles in particular (Barnes & Barnes 1965). Significant spatial and temporal variations in larval size at hatching among parents 500 have been recorded recently for A. modestus in our study area (Griffith 2013), but we still do 501 502 not know if these are carried over to the cyprid stage.

503 We conclude that trait-mediated effects can be important to understand the patterns of 504 recruitment of organisms to the adult cohorts. Early effects of the larval environment on post505 settlement survival can persist for months and eventually define number and quality of adults. Our data showed that this persistence was maintained through low levels of late juvenile 506 mortality occurring over the winter. Specific trait responses are central to understand the 507 nature of trait-mediated effects across gradients in thermal and nutritional stress. The key trait 508 responsible for the patterns of survival appears to be the size at metamorphosis, which may 509 affect the capacity to cope with food limitation or other stress during the first days of life. 510 Such a trait was shaped at the time of metamorphosis and did not fully correlate with larval 511 traits, which were also affected by larval nutritional conditions. We also conclude that trait-512 513 mediated effects can be context-dependent but that such phenomena also depend on the level of habitat harshness or the variability among cohorts in the phenotypic responses to 514 environmental conditions. 515

516

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TABLES

Table 1. *Austrominius modestus*. Two-way nested ANOVAs evaluating the effect of food concentration and replicate vessel (nested in food concentration) on body length of swimming cyprids for two different experiments. Significant effects are in bold. The F statistics of the food effect was calculated using the MS of the Vessel effect as denominator; the corresponding df was used for the calculation of the p value.

	Experiment 1						
	df MS		F	р			
Food	2	5003	17.0	<0.0001			
Vessel (F)	15	294	0.5	0.92			
Error	162	556					
	df	MS	F	р			
Food	2	48889	47.4	<0.0001			
Vessel (F)	14	1032	1.8	0.038			
Error	323	574					

Table 2. *Austrominius modestus*. One-way ANOVAs evaluating the effect of food concentration on dry mass (DW) and elemental composition (%C and %N) of swimming cyprids for Experiment 2 (degrees of freedom of food and error were 2 and 13 respectively). Significant effects are in bold.

	Dry	y mass (E	OW)	C:N ratio			
	MS	F p		MS	F	р	
Food	4.20	13.87	0.0006	3.10	13.50	0.0007	
Error	0.30			0.23			
		C (%)			N (%)		
	MS	F	р	MS	F	р	
Food	27.46	7.18	0.008	1.57	5.27	0.021	
Error	3.83			0.30			

Table 3. *Austrominius modestus*. Two-way ANOVAs evaluating the effect of food concentration on size of metamorphs (measured as basal and operculum length) out-planted at different intertidal levels during two different experiments. Significant effects are in bold.

Experiment 1		Ba	asal leng	th	Operculum length		
Experiment 1	df	MS	F	р	MS	F	р
Intertidal level	1	235	0.18	0.68	1816	1.52	0.23
Food	2	37029	27.67	<0.0001	8179	6.83	0.004
FxI	2	395	0.29	0.75	340	0.28	0.76
Error	27	1338			1198		
Exporimont ?		Basal length			Operculum length		
Experiment 2	df	MS	F	р	MS	F	р
Intertidal level	1	599	0.31	0.58	673	0.62	0.44
Food	2	10010	5.17	0.012	3769	3.50	0.043
FxI	2	1381	0.71	0.50	301	0.28	0.76
Error	30	1935			1078		

Table 4. *Austrominius modestus*. Three way repeated measures ANOVAs evaluating the effect of food concentration, intertidal level and time on cumulative barnacle survival for two different experiments. Significant effects are in bold.

	Experiment 1					Experiment 2			
_	df	If MS F F		р	df	MS	F	р	
Food	2	0.153	2.28	0.123	2	0.752	5.84	0.0082	
Intertidal level	1	1.271	18.93	<0.001	1	0.003	0.02	0.88	
FxI	2	0.259	4.81	0.035	2	0.05	0.41	0.67	
Error	25	0.067			25	0.129			
Time	5	0.282	57.45	<0.0001	5	0.665	153.96	<0.0001	
ТхF	10	0.004	1.315	0.52	10	0.007	1.65	0.10	
ТхІ	5	0.012	4.035	0.034	5	0.011	2.67	0.025	
ТхFхI	10	0.007	1.94	0.12	10	0.004	0.95	0.49	
Error	125	0.004			125	0.004			

Table 5. *Austrominius modestus*. Generalised Linear Models (GzLM) evaluating the effect of food concentration, intertidal level and time on barnacle growth (basal and operculum length) for two different experiments. Significant effects are in bold.

Exporimont 1		Basal le	ngth	Operculum	Operculum length		
Experiment I	df	χ^2 p		χ^2	р		
Intertidal level	1	16.78	<0.0001	17.79	<0.0001		
Food	2	0.57	0.75	1.14	0.56		
Time	4	404.93	<0.0001	375.80	<0.0001		
I x F	2	9.92	0.007	8.69	0.013		
S x T	4	13.88	0.008	7.87	0.096		
F x T	8	2.64	0.95	6.07	0.64		
IxFxT	8	4.06	0.85	9.44	0.31		
Experiment 2		Basal le	ngth	Operculum	Operculum length		
Experiment 2	df	χ^2 p		χ^2	р		
Intertidal level	1	7.59	0.006	7.46	0.006		
Food	2	1.84	0.40	2.95	0.23		
Time	5	613.94	<0.0001	562.78	0.0001		
I x F	2	1.85	0.40	0.45	0.80		
ΙxΤ	5	29.37	<0.0001	28.09	<0.0001		
F x T	10	27.05	0.0026	19.25	0.037		
IxFxT	10	3.09	0.98	2.24	0.99		

FIGURE LEGENDS

- Figure 1. *Austrominius modestus*. Effect of larval food concentration on body size of swimming cyprids. (a) Experiment 1. (b) Experiment 2. Food concentration: low (open bars), medium (striped bars), high (closed bars). Different letters indicate significant differences between treatments after SNK posthoc test; error bars represent standard errors among replicate vessels.
- Figure 2. *Austrominius modestus*. Effect of larval food concentration on dry mass and elemental composition (C and N content) of swimming cyprids. (a) Dry mass. (b) % Carbon. (c) % Nitrogen. (d) C:N ratio. Food concentration: low (open bars), medium (striped bars), high (closed bars). Symbols as in Fig. 1.
- Figure 3. *Austrominius modestus*. Effect of larval food concentration on body size (basal and operculum length) of out-planted metamorphs at the time of out-planting (day 0). (a) Experiment 1: basal length. (b) Experiment 2: basal length. (c) Experiment 1: operculum length. (d) Experiment 2: operculum length. Intertidal level: low (open bars), high (closed bars). Different letters indicate significant differences between treatments after SNK posthoc test; error bars represent standard errors among replicate vessels. Note that no differences between intertidal levels are presented showing that the sizes at metamorphosis were evenly distributed among intertidal levels.
- Figure 4. Austrominius modestus. Effect of larval food concentration and intertidal level on survival of settlers through time. (a) Experiment 1: low intertidal. (b) Experiment 1: high intertidal. (c) Experiment 2: low intertidal. (d) Experiment 2: high intertidal. Food concentration: LF: low (open circle), MF: medium (closed square), HF: high (closed triangle). Error bars represent standard errors among replicate vessels. For Experiment 1, SNK posthoc tests were run for week, food and intertidal level combinations. Different letters indicate significant differences each week among food treatments; n.s. indicates no significant difference. For Experiment 2, a SNK posthoc test was run after a main food effect (interactions were not significant), different letters (at the end) indicate overall differences between food treatments.
- Figure 5. Austrominius modestus. Effect of larval food concentration and intertidal level on growth (basal length) of settlers through time. (a) Experiment 1: low intertidal. (b) Experiment 1: high intertidal. (c) Experiment 2: low intertidal. (d) Experiment 2: high intertidal. Food concentration: LF: low (open circle), MF: medium (closed square), HF: high

(closed triangle). Error bars represent standard errors among replicate vessels. Different letters indicate significant overall differences among food treatments after 10 weeks after SNK posthoc test; n.s. indicates no significant difference.

Supplementary figure. *Austrominius modestus*. Standardised average (X) effect of food concentration on cyprid length, metamorph basal length, and survival after 2 weeks. For each variable, the averages were standardised separately following the equation: $\mathbf{X} = (\dot{\mathbf{X}}_i \cdot \ddot{\mathbf{X}})/\mathbf{S}$ with $\dot{\mathbf{X}}_i$ = mean value for each treatment ($\dot{\mathbf{X}}_1$, $\dot{\mathbf{X}}_2$, $\dot{\mathbf{X}}_3$), $\ddot{\mathbf{X}}$ = overall mean value ($\dot{\mathbf{X}}_1$, $\dot{\mathbf{X}}_2$, $\dot{\mathbf{X}}_3$), $\ddot{\mathbf{X}}$ = overall mean value ($\dot{\mathbf{X}}_1$, $\dot{\mathbf{X}}_2$, $\dot{\mathbf{X}}_3$), \mathbf{S} = standard deviation ($\dot{\mathbf{X}}_1$, $\dot{\mathbf{X}}_2$, $\dot{\mathbf{X}}_3$) and plotted using the same scale. Food concentration: LF: low (open bars), MF: medium (striped bars), HF: high (closed bars). The survival data from Experiment 1 were standardised only from low intertidal since there was no effect of food on the high intertidal. For Experiment 2 survival data from both intertidal levels were pooled since both levels showed the same effects of food concentration.









Experiment 1

A

MEDIUM

Food concentration

Low intertidal

High intertidal

A

LÓW

(C)

В

HIĠH

0.6

0.5

0.4

0.3

Operculum length (mm)

Experiment 2



Experiment 2











Supplementary figure