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Published in:
Marine Ecology-Progress Series

Publication date:
2016

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

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SAMS

25 KEYWORDS:

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ABSTRACT

One of the central issues in marine ecology is to identify processes affecting the structure and dynamics of populations with complex life cycles. The question of how larval processes affect recruitment has mostly focused on variations in settlement densities, with little yet known about how the larval environment determines post-settlement performance and recruitment to the adult stage. The objective of this work was to determine the role of trait-mediated effects of larval experience on recruitment under different environmental contexts. Using the intertidal barnacle *Austrominius modestus* as a model species, we manipulated the food concentration (*Skeletonema costatum* densities in cells ml^{-1}: low - $1x10^5$, medium- $2x10^5$, high- $3x10^5$) experienced by nauplius feeding stages and monitored patterns of survival and growth in out-planted settlers at two intertidal levels over a period of 5 months. The experiment was repeated twice in 2011 (September and October out-plants). In both experiments higher food levels resulted in increased cyprid size, dry mass, carbon and nitrogen content per individual and C:N ratio as well as increased body size of newly metamorphosed barnacles. In the September out-plant high food concentration reduced juvenile mortality at the low shore level while on the upper shore mortality was high irrespective of larval food concentration. By contrast in the October out-plant, larval food concentration affected juvenile survival at both shore levels. When present, differences in barnacle abundance due to larval conditions were established early (weeks 1 or 2) and persisted for long periods, over 10 or 22 weeks (September and October out-plants respectively). Interactive effects of the larval and juvenile environments can have important implications for population size: trait-mediated effects may persist for long periods contributing to explaining patterns of adult abundance.

INTRODUCTION

One of the central issues in ecology is to identify processes affecting the structure and dynamics of populations and communities. For species with complex life cycles, a better understanding of such processes is achieved if studies integrate both pre- and post-metamorphic stages (Thorson 1950, Grosberg & Levitan 1992, Caley et al. 1996, Connolly et al. 2001, Jenkins 2005, Allen & Marshall 2010). The nature of these life stages has important consequences for population dynamics and connectivity (Armsworth 2002, Shima & Swearer 2009). Pre-metamorphic stages (larvae) in terrestrial systems have restricted movements because they develop in landlocked aquatic environments (e.g. amphibians) or because of restricted locomotion capacity (e.g. insects). In contrast in marine systems, larvae often disperse widely (Strathmann 1990). Decoupling between local reproduction and input into the local population makes 'open' populations of many marine species with planktonic dispersal stages, resulting in fundamentally different dynamics to those of terrestrial 'closed' populations (Caley et al. 1996, Armsworth 2002). This is particularly important for benthic species, characterised by adults that are sessile or have restricted powers of locomotion. In marine systems, observations show that larval settlement rates can vary enormously over a number of spatial and temporal scales (e.g. Jenkins et al. 2000, Navarrete et al. 2002) as a result of both planktonic (physical transport, larval behaviour, larval mortality) and settlement processes. The consequences of such variation to adult population dynamics has been debated extensively (e.g. Connell 1985, Gaines & Roughgarden 1985, Menge 2000, Jenkins et al. 2008) with arguments for the strong role of pre-settlement processes in determining population structure (the recruitment limitation hypothesis of Doherty 1981) contrasting with those outlining a dominant role of post-settlement density dependent processes. A combined view of these extremes, represented by the recruit-adult hypothesis of Menge (2000) and extended by Jenkins et al. (2008), recognises a balance between pre- and post-settlement processes dependent on context and particularly the level of larval supply. In addressing these questions investigators have considered, almost without exception, density-mediated effects of the larval environment on juvenile survival (Giménez 2004), i.e. planktonic and settlement processes which directly affect the number of individuals which settle. However, there is increasing recognition that pre-settlement processes affect not only the number but also the traits of settling larvae (Giménez 2004, Pechenik 2006). Hence, the assumption adopted by empirical and modelling studies that all individuals to a given

83 population are equal is misleading as it overlooks the potentially large trait-mediated effects
84 of the larval environment on survival to the adult cohorts (Giménez 2004).

85 Understanding of trait-mediated effects of the larval environment on post-metamorphic
86 performance is increasing: it is clear that the larval environment can have a profound
87 influence on individual size and available reserves at settlement, which can determine
88 metamorphic success (e.g. Tremblay et al. 2007), survival (Pechenik et al. 2003) and the
89 ability to tolerate food limitation (Thiyagarajan et al. 2003 a,b), desiccation and wave action
90 (Phillips 2002) over the first days of post-metamorphic life. However, there is scarce
91 knowledge about the long term consequences of trait-mediated effects. Strong effects, i.e.
92 those that can influence population dynamics, should have long-term consequences on the
93 number of individuals reaching reproductive maturity or on fecundity. The strength of such
94 effects may be restricted by life history traits such as the length of the post-metamorphic
95 phase or by environmental conditions experienced during that phase. In species with a short
96 juvenile phase (<4 weeks) the larval environment can affect fecundity (Wendt 1998); extreme
97 cases are the holometabolous insects where feeding larvae eclose into an adult stage whose
98 energy reserves largely depend on larval history (Aguila et al. 2013). However, lack of long
99 term effects, on for example reproduction, has been observed in species with short life cycles
100 (e.g. Pechenik & Cerulli 1991). Environmental stochasticity may also limit the strength of
101 trait-mediated effects because it may blur the relationship between the larval environment,
102 post-metamorphic phenotype and survival. While laboratory studies, keeping environmental
103 conditions constant, show that effects of larval experience on phenotype are still found ca.
104 three months after metamorphosis (Giménez et al. 2004, Giménez 2010), relationships
105 between early and late phenotypes are sometimes weak in the wild (Lindholm et al. 2006,
106 Auer et al. 2010).

107 Context-dependent environmental conditions may also modify the strength of a trait-
108 mediated effect, but the limited number of studies still precludes the formulation of specific
109 predictions (Allen & Marshall 2013). In principle, trait-mediated effects may arise if
110 environmental conditions lead to differential levels of stress across phenotypes; such
111 conditions may be intermediate between a high stress environment where all die and low
112 stress where trait-mediated advantages are not expressed. However, field studies evaluating
113 larval responses to egg size and thermal conditions, i.e. across another life history boundary,
114 suggest that complex patterns are possible: in the frog *Bombina orientalis* larvae hatching
115 from large eggs perform better at low temperatures or under low variability in temperature

but the patterns reverse at high temperatures (Kaplan 1992, Kaplan & Phillips 2006). In marine species, observations of high mortality early after metamorphosis in a range of environmental contexts (Gosselin & Qian 1997, Hunt & Scheibling 1997, Underwood & Keough 2001, Gosselin & Jones 2010) suggest trait-mediated effects will appear early irrespective of the environment. However, the fact that mortality at advanced juvenile stages can be very high if intraspecific competition increases as individuals use more resources (Jenkins et al 2008, Giménez & Jenkins 2013) suggest that temporal changes in habitat quality can be important.

Intertidal acorn barnacles are a useful model system to address both density and trait-mediated effects on population dynamics. They develop through a series of pelagic larval feeding stages, the nauplius, followed by a non-feeding larval stage, the cyprid which settles and metamorphoses. Food conditions experienced by nauplius stages determine the amount of 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, b). Metamorphosis requires a considerable amount of total available reserves (e.g. 30% in *Semibalanus balanoides*: Lucas 1979) and feeding does not start until 2-5 days after metamorphosis (Rainbow & Walker 1977). Hence it is not surprising that both laboratory (Pechenik 1996) and short term field studies (Jarret 2003, Tremblay et al. 2007) have found that metamorphic success and early post-metamorphic survival are influenced by the larval food environment and positively correlate with the amount of cyprid reserves (Jarret & Pechenik 1997, Miron et al. 1999). However the long term effects of the larval environment and how this interacts with levels of post larval environmental stress are not known. We studied the effect of larval food environment on larval quality and subsequent long term post-metamorphic survival and growth of an intertidal acorn barnacle in two habitats characterised by different levels of environmental stress. By rearing larvae at three food densities and out-planting metamorphed juveniles barnacles at two intertidal levels, we exposed barnacles with different larval experience to different levels of desiccation, thermal and nutritional stress over a period of 22-25 weeks. The study addressed the following questions: (1) What is the relationship between the larval environment and the phenotype before and after metamorphosis? (2) Do we see trait-mediated effects? (3) If present, do trait-mediated effects depend on the environmental stress (tidal elevation)? (4) Are they restricted to a particular stage of development; (5) Do they propagate through time or alternatively does stochastic variation override the signal?

149

150 ## METHODS

151 The model species

152 *Austrominius modestus*, a non-native species originally from Australasia, was first
153 recorded in the UK in 1945 (Crisp 1958) and since then it has spread rapidly throughout the
154 European continent (Harms 1986). The early life cycle of *A. modestus* includes six naupliar
155 stages and a cyprid that settles in the rocky intertidal and metamorphoses to the first sessile
156 juvenile. Nauplii are planktotrophic stages, dependent on external food to grow and develop;
157 by contrast, cyprids do not feed. The duration of larval development depends on temperature:
158 in the Irish Sea, larvae are expected to take ca. 15 days (Harms 1999) to reach the first
159 juvenile stage. In the study area, larval development and settlement takes place mainly during
160 the summer through to early autumn. Juveniles feed on plankton at high tide, grow rapidly
161 and are able to breed within 12 weeks (Crisp & Davies 1955).

162 Laboratory and field procedures

163 Adult *Austrominius modestus* were collected from the mid intertidal zone of Menai Bridge
164 (Isle of Anglesey, UK), and maintained in the laboratory in seawater. In two separate
165 experiments in September and October 2011 larval release was stimulated by detaching the
166 adults from the rock. For each experiment freshly hatched larvae were divided among 18 5l
167 vessels. Nauplii were mass-reared at an initial density of 0.8-1.0 individual per ml at three
168 different food concentrations (6 replicate vessels per food treatment) using the diatom
169 *Skeletonema costatum* as food (Harms 1987). Larvae were reared following Harms (1987) at
170 low ($1x10^5$ cells ml^{-1}), medium ($2x10^5$ cells $ml^{-1)}$ and high ($3x10^5$ cells ml^{-1}) food
171 concentrations at 16ºC under gentle aeration. These concentrations produced low larval
172 mortalities in preliminary experiments. Water and food were changed every second day and
173 dead larvae discarded. Towards the end of each experiment, water was changed daily and
174 cultures were inspected for cyprids. When cyprids amounted to 50-80% of larvae present (in
175 most cases ca. 24-48 h from when the first cyprids were observed) the contents of each
176 culture vessel were transferred to a separate settlement vessel made of PVC each containing 6
177 natural slate tiles of 3x3 cm each. Cyprids of *A. modestus* may delay metamorphosis for up to
178 4-5 days, but observations over many trials showed that settlement peaks occurred within 48h
179 (Torres pers obs). Thus after 48 hours, tiles (with 10 or more settlers each) were out-planted

to the field and remaining swimming cyprids discarded, to avoid confounding food treatment effects with effects of delayed metamorphosis. We used the culture and settlement vessels as replicate unit, therefore all tiles originated from each vessel were considered as one replicate. A minimum of five vessels from each food treatment produced suitable tiles.

Tiles (2 to 3 from each vessel) were randomly fixed onto PVC frames using a 5mm pre-drilled hole through the tile centre. Frames carrying between 15 and 25 tiles were bolted to the natural rock (Experiment 1: 21st September 2011; Experiment 2: 17th October 2011) at an intertidal rocky outcrop under the suspension bridge in the Menai Strait (ca. 800m from the laboratory). Two different intertidal levels were used: the upper shore (4.8 m above Chart Datum) and low shore (3.0 m above Chart Datum) corresponding to the upper and lower distribution of barnacles. In total between 100 and 400 individuals were out-planted per treatment combination with a minimum of 10 individuals per tile.

All tiles were photographed before out-planting and then at weekly (week 1) and 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). During the first two weeks, tiles were photographed under a dissecting microscope (Leica Microscope MZ 6) by transporting tiles, attached to the PVC frame, to the laboratory during low tide, and returning before the 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 a PVC frame). Digital images were processed using Image J software; all surviving individuals were counted and the basal and operculum length measured in 5 individuals from each replicate settlement vessel. Measurements ended when less than 5 individuals per replicate vessel remained on the tiles (week 10 for Experiment 1 and week 22 for Experiment 2).

Body size, dry mass and elemental composition of swimming cyprids

In both experiments cyprid body size was determined by measuring 20 cyprids per replicate vessel under the microscope; cyprids were collected as swimming individuals within the first 48h of the first cyprids being observed. In Experiment 2, dry mass and elemental composition were also determined by sampling 100 swimming cyprids from each replicate vessel. Sample processing followed Anger & Harms (1990): 100 individual cyprids were pipetted out of each replicate vessel, quickly rinsed in distilled water, blotted dry with filter paper, placed in aluminium cartridges and frozen at -20C for later analysis; 20 randomly

chosen individuals per sample were measured under the microscope before being placed in the cartridges. Samples were freeze-dried (Edwards Supermodulyo 12 k freeze-drier) and weighed using a microbalance (Mettler Toledo, precision = 1 µg). Elemental composition (Carbon and Nitrogen content) was determined using a CHNS-O Analyser (Thermo Electron Flash EA 1112 Series).

Statistical analysis

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 shore level and larval food using a two-way ANOVA. While we expected an effect of larval food, we wanted to check that body size of metamorphs from each food treatment did not vary between shore levels at the time of out-planting. Our analysis confirmed that body size did not differ among shore levels at that time (see results).

The effects of larval food concentration, shore 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 test) and residuals did not show any serious deviations from the normal distribution.

The effects of larval food concentration, shore 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.

RESULTS

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

243 For both Experiments 1 and 2, larval food concentration significantly affected cyprid body
244 length (Table 1), with low food concentration resulting in a 4 to 7% reduction in size
245 compared with those from the high food level, and intermediate food concentration resulting
246 in an intermediate size (3% smaller) for Experiment 2 (Fig. 1).

247 Dry mass (DW) and elemental composition were only measured in Experiment 2. At low
248 food concentrations cyprid DW was 41% lower than at intermediate and high food levels
249 (Table 2: significant food effect, Fig. 2a). This reduction in overall mass was reflected in the
250 significant effect of food treatment on carbon (C) and nitrogen (N) content per individual
251 (data not shown). The amount of carbon per individual cyprid, for example, was 47% lower
252 at low food concentrations compared to high. Relative levels of C and N also responded to
253 food treatments. % C was significantly greater in the high food treatment with 9.5% and 7%
254 lower values in low and intermediate food treatments respectively (Table 2, Fig 2b). In
255 contrast to all other patterns % N showed the highest value in the low food treatment,
256 significantly greater than in the mid and high treatments (11-13% reduction, Fig. 2c). The
257 strong food effects on C compared to N led to a significant decline in the C:N ratio from high
258 to mid to low food treatments; in larvae reared under low food concentrations this ratio was
259 21% lower than in those reared under the highest food concentration (Fig. 2d).

260 At the time of out-planting body size (basal and operculum length) of metamorphs (within
261 24hs. of metamorphosis) varied among food concentrations but not between shore levels
262 (Table 3, Fig. 3) showing that individuals of different sizes were effectively allocated
263 randomly among shores. In both experiments, the highest food concentration resulted in the
264 largest basal length after metamorphosis (Fig. 3 a-b). Low food concentration resulted in
265 metamorphs that were 15% (Exp-1) and 8% smaller (Exp-2) in basal length than those from
266 high food concentration. The operculum length was largest for individuals metamorphosed
267 from larvae reared under high food concentrations in Experiment 1 (Fig. 3c), but similar sizes
268 were found between individuals reared under high and medium food concentrations in
269 Experiment 2 (Fig. 3d).

270 It is interesting to note in Experiment 1 the way in which cyprid size responded differently
271 to food treatments than metamorph size (Fig 1a vs Fig 3a). The medium food concentration
272 produced cyprids equivalent in body length to those at high food concentrations. However,
273 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.

Post-metamorphic survival

In both experiments the number of surviving barnacles 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 environment on survival depended on shore level and time after metamorphosis (Table 4, significant 3-way interaction). Significant effects of larval food concentration were restricted to the low shore: high larval food concentrations resulted in the highest survival; the differences between low and intermediate food concentrations were not significant. This effect of food on survival on the low shore was established between the time of out-planting and week 2 (06/10/11). On average 65% of metamorphs originated from the high food level survived the first two weeks after out-planting; only 37-46% of those from the intermediate and low food level survived that period (Fig. 4a, SNK posthoc tests). By contrast, in the high shore survival was low irrespective of the larval food treatment (on average 25% of the out-planted metamorphs, Fig. 4b).

The effect of food on survival in experiment 1, established in the low shore at week 2, remained until week 10 (30/11/11), except in week 6 (Fig. 4a, SNK posthoc test). During this period, bi-weekly survival rates were > 80% irrespective of food treatment. By week 10, the cumulative survival in the low shore was on average ca. 37% in juveniles metamorphosed from larvae fed at high food concentrations while those at intermediate and low food concentrations showed an average survival of 24 and 15%, respectively (Fig. 4a). At week 25 (16/03/12) the effect of larval food concentration on survival was not significant.

In Experiment 2 there was a significant main effect of larval food concentration which was consistent across both shore levels (Table 4); survival rates were lowest in metamorphs originated from the lowest food concentrations while those from intermediate and high food concentration showed similar levels of survival (Fig. 4 c-d, SNK posthoc test). In this experiment the effect of larval food conditions on survival was apparent one week (24/10/11) after settlement and remained over the whole 22 week study period. The percentage survival after a week was on average 49% for the metamorphs from the low food level and 66% for those from high and intermediate food levels. After the first week, survival rates were high (bi-weekly survival on average >78%) irrespective of food treatment.

306 Post-metamorphic growth

307 Overall, barnacles grew from ca. 0.55 to 4-5 mm in basal length (Fig. 5) and from 0.39 to
308 1.5-2.5 mm in operculum length (data not shown). In Experiment 1, the effect of food
309 concentration was found only in the low shore level (shore level x food interaction: Table 5):
310 high food concentration led to significantly larger body size on the low shore, (basal length,
311 Fig. 5a) and operculum length (not shown) than the intermediate and lower food
312 concentrations (SNK posthoc test). These differences were established at the time of out-
313 planting (see Fig. 3 for details) and appeared to increase with time (Fig.5a). Initially the basal
314 length of metamorphs from the low and intermediate food treatments were on average 0.12
315 mm smaller than those from the high food treatment (0.55 vs 0.67 mm); this difference
316 increased to 1mm after 10 weeks. By contrast, proportional differences varied little between
317 the time of out-planting (17%) and after 10 weeks (19%). There was no effect of food
318 treatment on growth in experiment 2.

319 **DISCUSSION**

320 Spatial and temporal variation in the arrival of new individuals is important in determining
321 the structure and dynamics of populations and communities (Gaines & Roughgarden 1985,
322 Caley et al 1996, Connolly et al. 2001, Jenkins et al 2008) and metapopulation persistence
323 (Armsworth 2002). However recent work shows that variations in the traits of organisms as
324 well as simply numbers also affect subsequent survival or reproduction (Pechenik 2006) and
325 can translate into effects on recruitment (Giménez 2004) and reproductive potential for a
326 population (Burgess & Marshall 2011). In species with complex life cycles and short
327 generation times effects of the larval environment on the size of adult cohorts may be likely
328 because the post-metamorphic life is short. However, it is not straightforward to expect
329 similar effects for species with longer post-metamorphic life because biotic interactions,
330 stress, disturbance or density-dependent effects on mortality or growth (Jenkins et al 2008)
331 experienced after metamorphosis may prevail over any effect produced by the pre-
332 metamorphic environment. Using an intertidal barnacle as a model we found (1) important
333 effects of the larval environment on the traits of settling barnacles; (2) that the effect of the
334 larval environment on post-settlement mortality and to a lesser extent growth was context-
335 dependent and short-lived but with consequences that lasted for a number of months,
336 affecting the abundance and size of individuals reaching adult stages. The magnitude and
337 duration of the trait-mediated effects were context-dependent: they varied in space and time

338 according to the post-metamorphic conditions. This context dependency adds to the complex
339 set of direct and indirect interactions existing in natural communities (see e.g. Menge 2000
340 for the rocky intertidal).

341 The effects of nauplius feeding history on cyprid traits were consistent with findings for other
342 barnacles (Thiyagarajan et al. 2002 b, Emlet & Sadro 2006). Low food concentration resulted
343 in a reduction in cyprid size, % C content and body mass (DW) as well as a reduced C:N
344 ratio. Thus our food treatments had a clear effect on a number of larval traits which were
345 then carried forward to post metamorphic performance. In linking larval traits with
346 performance later in life the direct relevance of particular measures is not entirely clear since
347 measures of size and biochemical indices are correlated (see Phillips 2002). Large larvae and
348 hence large juveniles may gain an advantage in stressful emersed conditions through a
349 relative reduction in surface area per unit volume, but equally size-associated energy reserves
350 are likely to be important in the critical transition from pelagic to benthic feeding modes.
351 The nature of available energy reserves such as the proportion of triacylglycerols have been
352 linked with variations in growth and survival of early life history stages in a range of taxa
353 including barnacles: Thiyagarajan et al. 2002 a,b; Tremblay et al. 2007 and bryozoans:
354 Wendt 1998). Our results are in line with the hypothesis that either larger size or higher level
355 of reserves, reflected by high C content and high values of C:N ratio confer higher fitness.

356 Observed effects of food treatment on size showed interesting differences between cyprids
357 (the end point of larval life) and early metamorphs; patterns in size among food treatments
358 were not equivalent between these two life stages. For example in experiment 1 intermediate
359 levels of food produced large cyprids which were equivalent in size to the high food
360 treatments, but metamorphs which were small and equivalent to individuals raised on low
361 food. A similar mis-match occurred in experiment 2. Examination of standardised average
362 values of cyprid and metamorph length and early survival show clearly that survival was
363 linked to metamorph, not cyprid size (Supplementary figure). The mechanism giving rise to
364 this effect is not clear but does emphasise the importance of early metamorph size on post
365 metamorphic performance.

366 An important output of our work is the finding that the effect of larval food environment on
367 post metamorphic performance varied across intertidal levels and between experiments
368 conducted at different times. Thus larval effects were context dependent. Variable effects of
369 tidal height on the consequences of larval food environment on post metamorphic

performance between the two experiments suggest that the level of emersion stress may be important in determining whether larval traits matter. Temperature records from a nearby meteorological station (Hilbre Island), 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 barnacles out-planted in October (13.2°C); in the same period, in September daily temperature maxima were above 25°C but maxima for October were 20°C. These data combined with the laboratory observations of Foster (1971) of 50% mortality rates of *A. modestus* recruits at 20°C suggest that high shore outplants in Experiment 1, where larval food treatment effects were not observed, will have received potentially much higher levels of emersion stress than those in experiment 2, where effects were clear. Looked at another way, in experiment 1 when temperature data suggest conditions were stressful, there was a difference in the expression of larval food treatment effects on both survival and growth between tidal heights (effect at low shore but not high), whereas in experiment 2 when conditions were potentially less stressful there was no difference (an effect on survival at both low and high shore).

These results point to the difficulty of considering how environmental conditions may mediate larval effects. Most related studies argue that the benefits of better quality larval phenotype will be expressed in poorer quality environments (e.g.REFS) yet we showed a reduction in expression with a reduction in quality of the environment (ie high shore hot conditions). Similar observations were made by Moran and Emlet (2001) who showed that hatching size of the gastropod *Nucella ostrina* positively affected early survival in a benign shaded habitat but not in a stressful sun-exposed environment. Thus the advantage of large hatching size decreased as environmental conditions declined in quality. We argue that intermediate levels of stress (or intermediate quality of the environment) are likely to promote the effect of larval environment on benthic performance. This is based on the assertion that too much stress and trait effects may be masked by overall high mortality, too little stress and the advantages gained by high quality larvae may not be expressed. We showed the greatest expression of larval effects in moderately benign intertidal conditions (Experiment 1 – only in the low shore during a hot period; Experiment 2- across both tidal levels during a cooler period). There have been no direct tests of whether intermediate stress will maximise larval effects and any such test (as for the intermediate disturbance hypothesis Connell 1978) would need to be performed across a wide range of stress levels. This is emphasised by considering studies where environmental modification of larval effects

403 has been examined but not found; for example Phillips (2002) found no difference in the
404 expression of larval nutritional history on juvenile mussel growth between sub-tidal and low
405 shore environments. Such lack of environmental effects could be argued be due to
406 differences in environmental quality which don't meet some critical level.

407

408 An alternative view of our results is that the different outcomes of the two experiments may
409 reflect variations in larval phenotypes among cohorts. In the cohort out-planted in October
410 the effect of food densities on basal and operculum diameter was weaker than in September.
411 Variations in phenotypes may reflect genetic variability or maternal effects on egg sizes and
412 embryonic development. Variations in egg size within populations are important in marine
413 invertebrates in general (Marshall & Keough 2008) and intertidal barnacles in particular
414 (Barnes & Barnes 1965). In crustaceans, variations in larval size at hatching can affect the
415 growth responses to food limitation (González-Ortegón & Giménez 2014) and size at
416 metamorphosis (Giménez et al. 2004). Significant variations in larval size at hatching among
417 parents have been recorded recently for *A. modestus* in our study area (Griffith 2013), but we
418 still do not know if these are carried over to the cyprid stage.

419 The persistence of trait mediated effects is a critical issue in considering the importance of
420 larval environment to population dynamics. While demonstration of the effects of larval
421 history on performance for days or weeks after settlement are common (REFS), longer term
422 studies are relatively rare (but see REF). We showed larval food treatment effects on survival
423 which persisted for between 10 and 22 weeks after settlement depending on the experiment
424 and the shore level, and effects on juvenile size after 10 weeks. Treatment effects on survival
425 and size were established within 2 weeks of settlement over the period when mortality in
426 invertebrate juveniles is known to be particularly high (Gosselin & Qian 1997, Hunt &
427 Scheibling 1997, Underwood & Keough 2001, Gosselin & Jones 2010). The quality of
428 larvae is probably of particular importance in the early benthic phase in barnacles since
429 during the first days after metamorphosis, barnacles cannot feed (Rainbow & Walker 1977)
430 and hence rely on accumulated reserves. The patterns established during the first two weeks
431 then persisted, although actual mortality rates did not vary among food treatments.
432 Mortality rates after the second week were an order of magnitude lower (average daily
433 instantaneous mortality rate = 0.003 d^{-1} for both experiments) than the mortality during the
434 first two weeks (average = 0.064 d^{-1} and 0.079 d^{-1} for experiment 1 and 2 respectively). Such
435 patterns may reflect ontogenetic changes in the sensitivity to environmental conditions

related to the development of for example feeding structures: in this sense it is likely there is a critical window where effects of larval experience on survival are highest. Although the effect of larval food environment only had effects on survival rate soon after settlement, the persistence of these effects from settlement in the autumn through to the spring time (an age at which A modestus can be reproductively mature REF) is critical in demonstrating that larval traits can have a strong influence on population level processes. The generality of such temporal persistence of larval effects is not clear; we are not aware of any study tracking cohorts of marine organisms for several months after manipulating the larval environment. Previous studies have shown important effects of the larval environment on adult cohorts in species with short maturation times (e.g. weeks: Wendt 1998). Our study shows that such effects can occur in species with a maturation time of several month and highlights the fact that early patterns of mortality caused by the larval environment may be maintained though time and affect the abundance of adult stages in spite of important post-settlement mortality.

SYNTHESIS

We conclude that trait-mediated effects can be important to understand the patterns of recruitment of organisms to the adult cohorts, but they are dependent on the interaction of the post-metamorphic environment and the traits defining the phenotype of the early juvenile stage. In particular, traits that are specific to an organism (e.g. dependence of reserves to successful metamorphosis, lack of feeding capacity during the first days) may be central to understand the nature of trait-mediated effects across gradients in thermal and nutritional stress. In addition, early effects of the larval environment on the post-settlement survival can persist for months and eventually define number of individuals reaching the adult cohorts. Our data show that the persistence is maintained through low levels of late juvenile mortality occurring over the winter. Further studies should evaluate the importance of trait-mediated effects across environmental gradients and particularly address the question of whether they are maximised at some intermediate stress level.

References

Aguila J R, Hoshizaki D K, Gibbs A G (2013). Contribution of larval nutrition to adult reproduction in *Drosophila melanogaster*. J Exp Biol 216(3):399-406.

Allen R M, Marshall D J (2010). The larval legacy: Cascading effects of recruit phenotype on ecological interactions. Oikos, 119:977-1983.

Allen R M, Marshall D J (2013). Phenotypic links among life-history stages are complex and context-dependent in a marine invertebrate: interactions among offspring size, larval nutrition, and post-metamorphic density. Funct Ecol 27(6):1358-1366.

Anger K, Harms J (1990). Elemental (CHN) and proximate biochemical composition of decapod crustacean larvae. Comp Biochem Physiol B 97:69-80.

Armsworth, P R (2002). Recruitment limitation, population regulation, and larval connectivity in reef fish metapopulations. Ecology 83: 1092-1104.Auer S K, Arendt J D, Chandramouli R, Reznick D N (2010). Juvenile compensatory growth has negative consequences for reproduction in Trinidadian guppies (*Poecilia reticulata*). Ecol Lett 13(8): 998-1007.

Barnes H, Barnes M (1965). Egg size, nauplius size, and their variation with local, geographical and specific factors in some common cirripedes. J Anim Ecol 34:391-402.

Bishop M W H (1947). Establishment of an immigrant barnacle in British coasted waters. Nature 159, 501-502.

Caley M J, Carr M H, Hixon M A, Hughes T P, Jones G P, Menge B A (1996). Recruitment and the local dynamics of open marine populations. Annu Rev Ecol Syst 27, 477-500.

Connell J H (1985). The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. J Exp Mar Biol Ecol 93(1):11-45.

Crisp D J, Davies P A (1955). Observations in vivo on the breeding of *Elminius modestus* grown on glass slides. J Mar Biol Ass UK 34:357-380.

Crisp D J (1958). The spread of *Elminius modestus* Darwin in north-west Europe. J Mar Biol Ass UK 37(2):483-520.

Doherty P J, Williams D McB (1988). The replenishment of coral reef fish populations. Oceanogr Mar Biol Annu Rev 26:487-551.

Emlet R B, Sadro S S (2006). Linking stages of life history: How larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*). Integr Comp Biol 46(3):334-346.

Foster B A (1971). Desiccation as a factor in the intertidal zonation of barnacles. Mar Biol 8:12-29.

Gaines S, Roughgarden J (1985). Larval settlement rate: A leading determinant of structure in an ecological community of the marine intertidal zone. P Natl Acad Sci 82(11):3707-3711.

Giménez L, Jenkins S R (2013). Combining traits and density to model recruitment of sessile organisms. PLoS ONE 8(3).

Giménez L (2004). Marine community ecology: the importance of trait-mediated effects propagating through complex life cycles. Mar Ecol Prog Ser 283:303-310.

Giménez L (2010). Relationships between habitat conditions, larval traits and juvenile performance in a marine invertebrate. Ecology 91:1401-1413.

Giménez L, Anger K, Torres G (2004). Linking life history traits in successive phases of a complex life cycle: effects of larval biomass on early juvenile development in an estuarine crab *Chasmagnathus granulata*. Oikos 104:570-580.

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

Gosselin L A, Jones L A (2010). Effects of solar radiation on barnacle settlement, early post-settlement mortality and community development in the intertidal zone. Mar Ecol Prog Ser 407:149-158.

Gosselin L A, Qian P-Y (1997). Juvenile mortality in benthic marine invertebrates. Mar Ecol Prog Ser 146:265-282.

Gosselin L A, Qian P-Y (1996). Early postsettlement mortality of an intertidal barnacle: a critical period for survival. Mar Ecol Prog Ser 135:69-75.

Griffith K (2013). Climate-driven changes in the recruitment success of marine invertebrates: The role of food supply and temperature. PhD Thesis. Bangor University, UK.

Grosberg R K, Levitan D R (1992). For adults only? Supply-side ecology and the history of larval biology. Trends Ecol Evol 7(4):130-133.

Harms J (1987). Energy budget for the larval development of *Elminius modestus* (Crustacea: Cirripedia). Helgolander Meeresunters 41:45-67.

Harms J (1999). The neozoan *Elminius modestus* Darwin (Crustacea, Cirripedia): Possible explanations for its successful invasion in European water, Helgolander Meeresunters 52:337-345.

Harms J (1986). Effects of temperature and salinity on larval development of *Elminius modestus* (Crustacea, Cirripedia) from Helgoland (North Sea) and New Zealand. Helgolander Meeresunters 40:355-376.

Hentschel B T, Emlet R B (2000). Metamorphosis of barnacle nauplii: Effects of food variability and a comparison with amphibian models. Ecology 81:3495-3508.

Hills J M, Thomason J C, Milligan J L, Richardson M (1998). Do barnacle larvae respond to multiple settlement cues over a range of spatial scales? Hydrobiologia 375/376: 101-111.

Hunt H L, Scheibling R E (1997). The role of early post-settlement mortality in recruitment of benthic marine invertebrates. Mar Ecol Prog Ser 155:269-301.

Jarret J N, Pechenik J (1997). Temporal variation in cyprid quality and juvenile growth capacity for the barnacle *Semibalanus balanoides*. Ecology 78:1262-1265.

Jarrett J N (2003). Seasonal variation in larval condition and postsettlement performance of the barnacle *Semibalanus balanoides*. Ecology 84:384-90.

Jenkins SR 2005 Larval habitat selection not larval supply determines settlement patterns and adult distribution in two chthamalid barnacles. Journal of Animal Ecology 74:893-904

Jenkins S R, Murua J, Burrows M T (2008). Temporal changes in the strength of density-dependent mortality and growth in intertidal barnacles J Anim Ecol 77:573-584.

Kaplan R H, Phillips P C (2006). Ecological and developmental context of natural selection: maternal effects and thermally induced plasticity in the frog *Bombina orientalis*. Evolution 60:142-156.

Kaplan R H (1992). Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. Ecology 73:280-288.

551 Lindholm A K, Hunt J, Brooks R (2006). Where do all the maternal effects go? Variation in
552 offspring body size through ontogeny in the live-bearing fish *Poecilia parae*. Biol Lett
553 2:586-589.

554 Lucas M I, Walker G, Holland D L, Crisp D J (1979). An energy budget for the free-
555 swimming and metamorphosing larvae of *Balanus balanoides* (Crustacea: Cirripedia).
556 Mar Biol 55:221-229.

557 Marshall D J, Keough M J (2004). Variable effects of larval size on post-metamorphic
558 performance in the field. Mar Ecol Prog Ser 279:73-80.

559 Marshall D J, Keough M J (2008). The relationship between offspring size and performance
560 in the sea. Am Nat 171:214-224.

561 Marshall D J, Bolton T F, Keough M J (2003). Offspring size affects the post-metamorphic
562 performance of a colonial marine invertebrate. Ecology 84: 3131-3137.

563 Marshall D J, Cook C N, Emlet R B (2006). Offspring size effects mediate competitive
564 interactions in a colonial marine invertebrate. Ecology 87:214-225.

565 Menge B A (2000). Top-down and bottom-up community regulation in marine rocky
566 intertidal habitats. J Exp Mar Biol Ecol 250:257-289.

567 Menge B A, Lubchenco J, Bracken M E S, Chan F, Foley M M, Freidenburg T L, Gaines S
568 D, Hudson G, Krenz C, Leslie H, Menge D N L, Russell R, Webster M S (2003). Coastal
569 oceanography sets the pace of rocky intertidal community dynamics. PNAS 100: 12229-
570 12234.

571 Miron G, Boudreau B, Bourget E (1999). Intertidal barnacle distribution: a case study using
572 multiple working hypotheses. Mar Ecol Prog Ser 189:205-219.

573 Moran A L, Emlet R B (2001). Offspring size and hatchling performance of an intertidal
574 gastropod under variable field conditions. Ecology 82:1597-1612.

575 Pechenik J A (2006). Larval experience and latent effects--metamorphosis is not a new
576 beginning. J Integr Comp Biol 47:1-11.

577 Pechenik J A, Rittschof D, Schmidt A R (1993). Influence of delayed metamorphosis on
578 survival and growth of juvenile barnacles *Balanus amphitrite*. Mar Biol 115:287-294.

579 Pechenik J A, Wendt D, Jarrett J (1998). Metamorphosis is not a new beginning: Larval
580 experience reduces rates of postlarval growth, development, and survival in marine
581 invertebrates. BioScience 48:901-910.

582 Pechenik J A, Estrella S, Hammer K (1996). Food limitation stimulates metamorphosis and
583 alters postmetamorphic growth rate in the marine prosobranch gastropod *Crepidula*
584 *fornicata*. Marine Biol. 127:267-275.

585 Phillips N E (2002). Effects of nutrition-mediated larval condition on juvenile performance in
586 a marine mussel. Ecology 83:2562-2574.

587 Rainbow P S, Walker G (1977). The functional morphology of the alimentary tract of
588 barnacles (Cirripedia: Thoracica). J Exp Mar Biol Ecol 28(2):183-206.

589 Relyea R A (2001). The lasting effects of adaptive plasticity: predator-induced tadpoles
590 become long-legged frogs. Ecology 82, 1947-195.

591 Shima J S, Swearer S E (2009). Larval quality is shaped by matrix effects: implications for
592 connectivity in a marine metapopulation. Ecology 90(5):1255-1267.

593 Strathmann R (1990). Why life histories evolve differently at sea. Amer Zool 30,197-207.

594 Taylor B W, Anderson C R, Peckarsky B L (1998). Effects of size at metamorphosis on
595 stonefly fecundity, longevity, and reproductive success. Oecologia 114, 494-502.

596 Thiyagarajan V, Harder T, Qian P-Y (2002a). Effect of the physiological condition of cyprids
597 and laboratory-mimicked conditions on the metamorphic success of *Balanus amphitrite*
598 Darwin (Cirripedia; Thoracica). J Exp Mar Biol Ecol 274:65-74.

599 Thiyagarajan V, Harder T, Qian P-Y (2002b). Relationship between cyprid energy reserves
600 and metamorphosis in the barnacle *Balanus amphitrite* Darwin (Cirripedia; Thoracica). J
601 Exp Mar Biol Ecol 280:79-93.

602 Thiyagarajan V, Harder T, Qian P-Y (2003a). Effects of TAG/DNA ratio and age of cyprids
603 on post-metamorphic growth and survival in the barnacle *Balanus amphitrite*. J Mar Biol
604 Assoc UK 83:83-88.

605 Thiyagarajan V, Harder T, Qiu J W, Qian P-Y (2003b). Energy content at metamorphosis and
606 growth rate of the juvenile barnacle *Balanus amphitrite*. Mar Biol 143:543-554.

607 Thiyagarajan V, Soo L, Qian P-Y (2005). The role of sediment organic matter in larval
608 habitat selection by the polychaete *Capitella sp*. J Exp Mar Biol Ecol 323:70-83.

609 Thiyagarajan V, Pechenik J A, Gosselin L, Qian P-Y (2007). Juvenile growth in barnacles:
610 combined effect of delayed metamorphosis and sub-lethal exposure of cyprids to low
611 salinity stress. Mar Ecol Prog Ser 344:173-184.

612 Thiyagarajan V (2010). A review on the role of chemical cues in habitat selection by
613 barnacles: new insights from larval proteomics. J Exp Mar Biol Ecol 392:22-36.

614 Tremblay R, Olivier F, Bourget E, Rittschof D (2007). Physiological condition of *Balanus*
615 *amphitrite* cyprid larvae determines habitat selection success. Mar Ecol Prog Ser 340:1-
616 8.

617 Underwood A J, Keough M J (2001). Supply-side ecology: the nature and consequences of
618 variations in recruitment of intertidal organisms. In: Burtness MD, Gaines SD, Hay ME
619 (eds) Marine community ecology. Sinauer Associates, Sunderland, MA, p 183-200.

620 Van Allen B G, Briggs V S, McCoy M W, Vonesh J R (2010). Carry-over effects of the
621 larval environment on post-metamorphic performance in two hylid frogs. Oecologia
622 164(4):891-898.

623 Wendt D E (1998). Effect of larval swimming duration on growth and reproduction of
624 *Bugula neritina* (Bryozoa) under field conditions. Biol Bull 195:126-35.

625 West T L, Costlow J D (1987). Size regulation in larvae of the crustacean *Balanus eburneus*
626 (Cirripedia: Thoracica). Mar Biol 96:47-58.
627

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.

	Experiment 1				Experiment 2			
	df	MS	F	p	df	MS	F	p
Food	2	5003	13.9	**<0.0001**	2	48889	47.4	**<0.0001**
Vessel (F)	15	294	0.5	0.92	14	1032	1.8	**0.038**
Error	162	556			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 mass (DW)			C:N ratio		
	MS	F	p	MS	F	p
Food	4.20	13.87	**0.0006**	3.10	13.50	**0.0007**
Error	0.30			0.23		

	C (%)			N (%)		
	MS	F	p	MS	F	p
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 shore level during two different experiments. Significant effects are in bold.

Experiment 1		Basal length			Operculum length		
	df	MS	F	p	MS	F	p
Shore level	1	235	0.18	0.68	1816	1.52	0.23
Food	2	37029	27.67	**<0.0001**	8179	6.83	**0.004**
FxS	2	395	0.29	0.75	340	0.28	0.76
Error	27	1338			1198		

Experiment 2		Basal length			Operculum length		
	df	MS	F	p	MS	F	p
Shore level	1	599	0.31	0.58	673	0.62	0.44
Food	2	10010	5.17	**0.012**	3769	3.50	**0.043**
FxS	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, shore level and time on barnacle survival for two different experiments. Significant effects are in bold.

	Experiment 1				Experiment 2			
	df	MS	F	p	df	MS	F	p
Food	2	0.174	2.51	0.099	2	0.604	4.30	**0.024**
Shore level	1	1.729	24.92	**<0.0001**	1	0.040	0.29	0.59
FxS	2	0.333	4.81	**0.017**	2	0.109	0.77	0.47
Error	27	0.069			26	0.141		
Time	5	0.264	57.45	**<0.0001**	5	0.612	142.33	**<0.0001**
T x F	10	0.006	1.315	0.23	10	0.006	1.42	0.18
T x S	5	0.018	4.035	**<0.002**	5	0.009	2.19	0.059
T x F x S	10	0.009	1.94	**0.045**	10	0.005	1.20	0.29
Error	135	0.004			130	0.004		

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

Experiment 1		Basal length		Operculum length	
	df	χ^2	p	χ^2	p
Shore 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**
SxF	2	9.92	**0.007**	8.69	**0.013**
SxT	4	13.88	**0.008**	7.87	0.096
FxT	8	2.64	0.95	6.07	0.64
SxFxT	8	4.06	0.85	9.44	0.31

Experiment 2		Basal length		Operculum length	
	df	χ^2	p	χ^2	p
Shore 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**
SxF	2	1.85	0.40	0.45	0.80
SxT	5	29.37	**<0.0001**	28.09	**<0.0001**
FxT	10	27.05	**0.0026**	19.25	**0.037**
SxFxT	10	3.09	0.98	2.24	0.99

668 FIGURE LEGENDS

669

670 Figure 1. *Austrominius modestus*. Effect of larval food concentration on body size of
671 swimming cyprids. (a) Experiment 1. (b) Experiment 2. Different letters indicate
672 significant differences between treatments after SNK posthoc test; error bars represent
673 standard errors among replicate vessels.

674 Figure 2. *Austrominius modestus*. Effect of larval food concentration on dry mass and
675 elemental composition (C and N content) of swimming cyprids. (a) Dry mass. (b) %
676 Carbon. (c) % Nitrogen. (d) C:N ratio. Symbols as in Fig. 1.

677 Figure 3. *Austrominius modestus*. Effect of larval food concentration on body size (basal and
678 operculum length) of out-planted metamorphs at the time of out-planting (day 0). (a)
679 Experiment 1: basal length. (b) Experiment 2: basal length. (c) Experiment 1: operculum
680 length. (d) Experiment 2: operculum length. Different letters indicate significant
681 differences between treatments after SNK posthoc test; error bars represent standard errors
682 among replicate vessels. Note that no differences between shore levels are presented
683 showing that the sizes at metamorphosis were evenly distributed among shore levels.

684 Figure 4. *Austrominius modestus*. Effect of larval food concentration and shore level on
685 survival of settlers through time. (a) Experiment 1: low shore. (b) Experiment 1: high
686 shore. (c) Experiment 2: low shore. (d) Experiment 2: high shore. Error bars represent
687 standard errors among replicate vessels. Food concentration: LF: low, MF: medium, HF:
688 high. For Experiment 1, SNK posthoc tests were run for week, food and shore height
689 combinations. Different letters indicate significant differences each week among food
690 treatments; n.s. indicates no significant difference. For Experiment 2, a SNK posthoc test
691 was run after a main food effect (interactions were not significant), different letters (at the
692 end) indicate overall differences between food treatments.

693 Figure 5. *Austrominius modestus*. Effect of larval food concentration and shore level on
694 growth (basal length) of settlers through time. (a) Experiment 1: low shore. (b) Experiment
695 1: high shore. (c) Experiment 2: low shore. (d) Experiment 2: high shore. Error bars
696 represent standard errors among replicate vessels. Food concentration: LF: low, MF:
697 medium, HF: high. Different letters indicate significant overall differences among food
698 treatments after 10 weeks after SNK posthoc test; n.s. indicates no significant difference.