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Griffith, Kate; Jenkins, Stuart; Gimenez Noya, Luis

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Zoology

LARVAL TOLERANCE TO FOOD LIMITATION IS STRONGER IN AN EXOTIC BARNACLE THAN IN ITS NATIVE COMPETITOR --Manuscript Draft--

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Corresponding Author:	Luis Gimenez Bangor University Menai Bridge, UNITED KINGDOM		
First Author:	Luis Gimenez		
Order of Authors:	Luis Gimenez		
	Kate Griffith		
	Stuart R. Jenkins		
Abstract:	A critical question in marine ecology is understanding how organisms will cope with environmental conditions under climate change. Increasing temperatures not only have a direct effect on marine organisms but may also lead to food limitation through for example trophic mismatches, or by the increased metabolic demands imposed by developing at high temperatures. Using barnacles from a population of North Wales, we studied the combined effect of temperature and food density on the survival, settlement success, developmental time and body size of larvae of the native barnacle Semibalanus balanoides and its exotic competitor, the barnacle Austrominius modestus. Larvae were reared at similar food levels but at temperature ranges which varied among species reflecting their different phenology and tolerances. For S. balanoides (spring larval release) we used a lower temperature of 9 °C, reflecting spring temperatures from N Wales to SW England, and 15°C representing warmer conditions; for A. modestus (summer larval release) a typical summer temperature for this geographic range of 15°C was used with a raised temperature of 18°C. Larvae were reared under controlled conditions in automated, computer programmable incubators and fed diatoms (Skeletonema costatum) at three food levels. We found stress effects of food limitation on larval performance of S. balanoides. While survival during naupliar development was little affected by food and temperature, low food levels strongly depressed survival and settlement during the cyprid stage of S. balanoides at both tested temperatures, but especially at 15°C. By contrast, at the tested temperatures little effects were found on survival and settlement success in the expirid stage. Response in body size and development in response to low food levels while S. balanoides cyprids showed decreased body size at the high tested temperature. The main impact occurred as a delayed effect, at the time when cyprids attempt to settle, rather than as an effect on naupliar survival or metamorph		
Suggested Reviewers:	David Whethey University of South Carolina wethey@biol.sc.edu Expertise in ecology of marine barnacles Louise Firth, Dr Lecturer in Marine Biology, University of Plymouth louise.firth@plymouth.ac.uk		
	Expense in manne ecology and climate change, especially in focky shore organisms		

	Mark Johnson Professor, Galway University mark.johnson@nuigalway.ie Expertise in population ecology with emphasis on intertidal organisms
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Helgoland 31.11.2020

Dear Editor,

Please, find the second revised version of the manuscript entitled "Larval tolerance to food limitation is stronger in an exotic barnacle than in its native competitor". We have covered all the minor corrections indicated by the editorial office. We are now submitting the ms in separated files as requested.

Please, do not hesitate to contact me if you have any question.

Sincerely,

Dr. Luis Giménez Biologische Anstalt Helgoland, Alfred Wegener Institute, Helmholtz Zentrum für Polar and Meeresforschung 27498 Helgoland Deutschland Larval tolerance to food limitation is stronger in an exotic barnacle than in its native competitor. Kate Griffith, Stuart R. Jenkins and Luis Giménez

Highlights

- Under current warming, cold adapted native barnacle *Semibalanus balanoides* competes with warm adapted exotic *Austrominius modestus*
- Food limited larval performance is strong at high temperatures for native *S. balanoides*
- At similar temperatures and food density, larval performance of the exotic *A. modestus* is little affected.

1	LARVAL TOLERANCE TO FOOD LIMITATION IS STRONGER IN AN EXOTIC
2	BARNACLE THAN IN ITS NATIVE COMPETITOR
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4	Kate Griffith ¹ , Stuart R. Jenkins ¹ and Luis Giménez ^{1,2}
5	
6 7	 School of Ocean Sciences, Bangor University, Askew Street, LL59 5AB, Anglesey, Wales, United Kingdom.
8	2. Marine Station of Helgoland, Alfred Wegener Institute, Helmholtz Centre for Polar and
9	Marine Research, Ostkaje 1118, DE-27498 Helgoland, Germany.
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27 Abstract

A critical question in marine ecology is understanding how organisms will cope with 28 29 environmental conditions under climate change. Increasing temperatures not only have a direct effect on marine organisms but may also lead to food limitation through for example trophic 30 31 mismatches, or by the increased metabolic demands imposed by developing at high temperatures. 32 Using barnacles from a population of North Wales, we studied the combined effect of temperature 33 and food density on the survival, settlement success, developmental time and body size of larvae 34 of the native barnacle Semibalanus balanoides and its exotic competitor, the barnacle Austrominius 35 *modestus*. Larvae were reared at similar food levels but at temperature ranges which varied among species reflecting their different phenology and tolerances. For S. balanoides (spring larval 36 37 release) we used a lower temperature of 9 °C, reflecting spring temperatures from N Wales to SW England, and 15°C representing warmer conditions; for A. modestus (summer larval release) a 38 39 typical summer temperature for this geographic range of 15°C was used with a raised temperature 40 of 18°C. Larvae were reared under controlled conditions in automated, computer programmable 41 incubators and fed diatoms (Skeletonema costatum) at three food levels. We found stress effects 42 of food limitation on larval performance of S. balanoides. While survival during naupliar development was little affected by food and temperature, low food levels strongly depressed 43 survival and settlement during the cyprid stage of S. balanoides at both tested temperatures, but 44 45 especially at 15°C. By contrast, at the tested temperatures little effects were found on survival and 46 settlement success in the exotic A. modestus. Both species delayed development in response to 47 low food levels while S. balanoides cyprids showed decreased body size at the high tested temperature. The main impact occurred as a delayed effect, at the time when cyprids attempt to 48 49 settle, rather than as an effect on naupliar survival or metamorphosis to the cyprid stage. Response 50 in body size and developmental time may have costs at the time of metamorphosis (delayed settlement) or after metamorphosis. Overall, our experiments suggest that as temperature 51 increases, settlement success of S. balanoides larvae (but not that of its competitor A. modestus) 52 53 will become more sensitive to conditions of food limitation, imposed for instance by phenological mismatches with periods of phytoplankton peak. 54

55 **Keywords:** cirripedia - larvae - multiple stressor – warming

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57 **1. Introduction**

A key current question in ecology concerns the action of multiple climate driven variables on 58 59 organisms, populations and communities. Climate change involves a modification of a large number of environmental variables which can act simultaneously, potentially altering performance 60 61 and fitness (Crain et al., 2008; Hoffmann and Sgro, 2011; Piggott et al., 2015). In addition, climate 62 driven environmental variables can provide a novel context for how natural variation in limiting factors are handled by organisms (Spitzner et al., 2019). For example, in the marine habitat, larval 63 stages of crustaceans and fish are thought to experience a food limited environment (Barnes, 1956; 64 65 Olson and Olson, 1989; Gotceitas et al., 1996; Gimenez and Anger, 2005; Le Pape and Bonhommeau, 2015). In seasonal habitats, increases in temperature can be accompanied by 66 67 reductions in food availability because of phenological mismatches, between periods of larval development and that of food production (Edwards and Richardson, 2004). In addition, and 68 69 irrespective of changes in food availability, increased temperature results in increased metabolic 70 demands and "cost of living" (Somero, 2002). Those costs are likely to exacerbate effects of food 71 limitation on survival, development and growth (Giebelhausen and Lampert 2001; González-72 Ortegón and Giménez 2014; Torres and Giménez 2020).

73 Another concern is that climate change has been accompanied by range expansion or introduction 74 of exotic, potentially warm tolerant, species (e.g. Hickling, et al., 2006; Burrows et al., 2011; Poloczanska et al., 2013), which can produce important modifications in natural communities if 75 they become invasive (Simberloff, 2009; Gurevitch et al., 2011). In regions where warming is 76 accompanied by the arrival of exotic species, the overall result is that native species are faced with 77 harsher habitats but also need to invest extra resources in competing with exotic species. An 78 79 important point is however that the exotic species may have to cope with similar levels of food 80 limitation and increased temperature as the natives. Hence, the overall effect of increased 81 temperature, nutritional limitation and competition will depend on how both native and exotic competitor fare in the light of warming. 82

Here, we study a system of competing barnacles composed of a native species (*Semibalanus balanoides*) that is facing warming and the presence of an exotic competitor (*Austrominius modestus*), introduced from New Zealand (Crisp, 1958; Harms, 1987). Both species co-occur as

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juvenile-adults in rocky intertidal zones of North Europe (Crisp and Davies, 1955; Harms, 1982; 86 Torres et al., 2016) while larvae develop and grow in open coastal waters. The barnacle S. 87 *balanoides* releases a single brood of pelagic larvae during the spring (Barnes, 1956, 1957a, 1962); 88 89 A. modestus, however, releases larvae over spring and summer (Crisp and Davies, 1955). Our 90 study focuses on the larval phase because available information on success and failure of recruitment suggest that, at least in S. balanoides, larvae are quite sensitive to natural variations in 91 92 food availability (Barnes, 1956; Hawkins and Hartnoll, 1982; Cole et al., 2011) and because changes in larval physiological quality can drive settlement success and post-metamorphic survival 93 (Jarret, 2003). Combined negative effects of high temperature and food limitation may occur for 94 instance after warm winters, which are known to impact recruitment in S. balanoides (Polaczanska 95 et al., 2008; Abernot-Le Gac et al., 2018). More generally, larvae tend to be highly sensitive to 96 environmental fluctuations (Pandori and Sorte, 2019) and make a strong contribution to the 97 recovery and persistence of marine populations following disturbance (Cowen and Spounagle, 98 2009; Pineda et al., 2009; Gimenez et al., 2020). We therefore quantify the effect of food level and 99 temperature on survival of larvae, cyprid size, rate of development and the subsequent 100 101 metamorphic success. While larval survival and metamorphic success provide an indicator of effects that are relevant in the context of larval settlement rates, changes in development time and 102 103 body size, point towards potential trait-mediated effects (Torres et al., 2016; Gimenez et al., 2017) whereby changes in physiological traits driven by larval experience affect post-metamorphic 104 105 survival. We predict that S. balanoides, with larval release synchronised to phytoplankton blooms (Barnes, 1956, 1962; Hawkins and Hartnoll, 1982; Starr et al., 1991), should show lower tolerance 106 107 to low food levels than A. modestus, which does not synchronise its larval release to an environmental cue. We also predict that the ability of both species to tolerate low food conditions 108 109 will be impaired at species-specific high temperature levels.

110 2. Materials and Methods

111 2.1 Experimental design

Larvae hatched from broods produced by different parents were exposed to combinations of two temperatures and three food levels in a factorial design (Fig. 1). Because the model species differ in their temperature optima (Harms, 1987), we evaluated the role of food limitation at speciesspecific temperatures (*S. balanoides*: 9 °C and 15 °C; *A. modestus*: 15 °C and 18 °C) ensuring 116 mortality rates < 25% at optimal food conditions (Harms, 1987), but enabling direct comparison of responses at one of the temperature treatments. Our choice of temperature is based on those 117 experienced by larvae released from barnacles over the geographic extent of SW Great Britain, N. 118 France and Spain, during the period of larval release and development (spring for S. balanoides, 119 120 summer for A. modestus). Thus 9 °C represents spring time temperatures under which S. balanoides typically develops, with 15 °C used to represent particularly warm conditions. For A. 121 122 modestus which breeds throughout the year but with predominant larval development in summer months, 15 and 18 °C were used to represent normal and elevated conditions respectively. 123

Larvae were fed the diatom Skeletonema costatum which is known to promote development in 124 both species (Moyse, 1963; Tighe-Ford, 1970; Barker, 1976; Harms, 1987; Stone, 1989). There 125 were three food levels: high food (HF): 4 x 10⁵; mid food (MF): 2 x 10⁵ and low food (LF): 4 x 126 10⁴ cells ml⁻¹. The two highest food levels were within the range used by Harms (1987) and Stone 127 (1989). A preliminary experiment (Supplementary data: Fig. S1) showed us that the choice of food 128 treatments and a temperature of 15°C to perform direct comparisons among species, leds to 129 successful survival and settlement for both species. For each experiment, four adults with ripe 130 embryos were selected; larvae from each adult were reared under the 2 different temperatures for 131 132 each of the 3 experimental food levels. In summary, for the main experiment we used a total of 120 replicate units (= 5 units x 3 food levels x 2 temperature levels x 4 adults) and 1200 larvae (= 133 134 10 larvae x 120 replicate units) per barnacle species. For the preliminary experiment we also used 135 10 larvae per replicate unit and a total of 120 replicate units and 1200 larvae per species, but organised in 5 units x 3 food levels x 2 shores x 4 barnacles per shore. 136

137 2.2. Collection of animals and culturing procedures

Experiments were carried out in February 2012 for S. balanoides and in October 2012 for A. 138 modestus (preliminary experiment in April-July 2010). For each experiment, adults were collected 139 from a location on the Menai Strait (53° 13'16N, 04° 09'47W) and left to hatch. Adult barnacles 140 of S. balanoides and A. modestus were collected by scraping intact individuals from the 141 substratum. Upon return to the laboratory, adults that contained broods of ripe embryos were 142 143 identified and placed individually in separate 100ml plastic containers containing 0.45 µm filtered seawater (FSW). These were left for up to 2 hours to allow hatching of stage I nauplii (Crisp, 144 145 1956). Only adults where most of the larval brood had readily hatched were deemed appropriate

for experimental use and, from these, four adults were randomly selected for the experiment. Larvae were transferred to experimental culture vessels (10 larvae per 100ml vessel). All replicates were reared in automated incubators with a 12:12h light: dark photoperiod at the designated experimental temperature.

Diatoms Skeletonema costatum (strain: CCAP 1077/5) were provided at appropriate cell densities 150 every 2 days with provision of algae coinciding with the time of water change. Prior to each 151 152 feeding, S. costatum were collected from cultures (grown in Conway medium with added silicates 153 and FSW; Laing, 1991) and culture cell density was determined with a haemocytometer. Algal 154 cultures were reared in 4 or 10 litre Pyrex borosilicate flasks at 18-19°C with a 24 hr light cycle. Larval culture water was changed by pouring the contents of a replicate jar into a small glass bowl 155 156 and, using a Pasteur pipette, larvae were moved to a watch glass for microscopic examination. 157 After examination, larvae were returned to a clean jar already containing S. costatum and FSW at 158 the appropriate experimental temperature and food density. During microscopic examination, 159 larvae were checked for mortality and for average larval development stage (naupliar stages I-VI: 160 Crisp, 1962; Knight Jones and Waugh, 1949). Larvae within each replicate were observed every 2 days until the first appearance of cyprids, after which time, larval cultures were checked daily 161 162 (with water change and feeding still taking place every 2 days). All cultures ran until larvae had either died or moulted to the final larval cyprid stage. When the cyprid stage was reached, cyprids 163 164 were removed from cultures and subsequently used to determine settlement rates.

165 When larvae had developed to the cyprid stage, the cyprids from each adult reared under each food 166 treatment and temperature condition were pooled together in 1 jar. Cyprids were kept in the incubator in FSW at the same temperature they were reared under. Ten cyprids from each adult at 167 each treatment were selected at random and photographed using a microscope camera GX-CAM 168 5. Photographs were subsequently analysed using Image J to calculate the length, width and cyprid 169 170 area. Cyprids were then returned to the cultures for settlement rates analysis. For this, a settlement 171 surface was introduced to the vessel containing the cyprids and the resulting settlement rates were observed. Clean rock scraped from conspecific barnacles were provided as a settlement surface. 172 173 On day 2, 4, 6 and 8 (after the rock had been added) the cyprid cultures were examined and the water was changed. During examination, numbers of metamorphosed cyprids upon the rocks were 174 175 counted and any dead cyprids in the jar were removed.

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177 2.3 Data analysis

The instantaneous mortality rates (M) of larvae during naupliar development (hatching to cyprid) and cyprid settlement were calculated as $M = \ln (N_0/N_t)/-t$, where t = specific time interval; $N_t =$ number of larvae after a specific time interval (t); and N_o = initial number of larvae. Using this method, the average M for each food and temperature treatment were calculated for both the period of hatching to cyprid and the subsequent cyprid to settlement period for each species.

Statistical analyses were run in R (R Core Team 2013), using model selection based on the adjusted Akaike information criteria (AICc), using backward removal, following Zuur et al. (2009) and Galecki and Burzykowski (2013). The response variables were duration of development from hatching to the cyprid stage per replicate, percentage survival to cyprid per replicate, percentage settlement and mean cyprid size; all these variables were analysed through mixed modelling, using the package nlme (Pinheiro et al., 2018). Student Newman Keul's (SNK) was used for *post-hoc* multiple comparisons.

For survival and developmental time to cyprids, we fitted models containing random terms (based 190 on parent of origin) as well as fixed terms (i.e. effects of temperature and food on average trends), 191 192 using the *lme* function. Model selection for the random terms were carried out through restricted maximum likelihood (REML). Once the best random structure was fitted, we used maximum 193 194 likelihood (ML) for model selection on the fixed structure. For settlement success we had a single replicate per parent; therefore, we fitted models, using the gls function, based on a within subject-195 design (where parents were the subjects), by adding a correlation term (corCompSymm constructor 196 function). 197

Estimates of variance components were used to evaluate patterns of variability in the response variables among random factors (shore, parent and replicate) for each experimental treatment. The relative variance components were estimated for each source of variation by using the observed mean squares to estimate terms identified in the expected mean squares (Searle et al. 1992; Underwood, 1997; Graham and Edwards, 2001). Sometimes one or more estimates from the ANOVA method were negative; then, these estimates were set to zero, removed from the model and the estimates for the remaining factor re-calculated according to Fletcher and Underwood 205 (2002). Untransformed data were used throughout to provide variance components comparable206 across all data (Fraschetti et al. 2005).

207 **3. Results**

208 *3.1 Naupliar survival and settlement rates*

209 For both S. balanoides and A. modestus, survival to cyprid was high (>80%) under all of the experimental treatments (Fig. 2a, b); hence, effects of food limitation or temperature on survival 210 211 were weak. For S. balanoides, the best models did not include food or temperature as main factors driving survival to the cyprid stage (Table 1). The effect of food and temperature on survival rates 212 213 of S. balanoides varied among individuals hatching from different parents (best model included parent of origin in their random part: supplementary material: Table S1). Most important variation 214 215 among parents of S. balanoides occurred at the high temperature, especially at low food levels (supplementary material: Fig. S2). For A. modestus, the best models retained food and temperature 216 217 but the actual effect was weak, as survival was very high, >90%, under all experimental conditions (Fig. 2b). Post-hoc tests on averages by parent did not detect differences among treatments and 218 variance components indicated low contribution of parents to the total variability for this species 219 (supplementary material: Fig. S2). 220

Settlement rates were more responsive to food condition in S. balanoides than in A. modestus (Fig. 221 3a, b). In S. balanoides, both temperature and food condition were retained in the best statistical 222 223 models (Tables 1 and S2 for details) but the effect of temperature was weak. Settlement was reduced under low food levels (Fig. 3a) especially at the high temperature treatment (15° C), with 224 only 48% of the larvae settling compared to 77% at 15 °C for the mid and high food (no significant 225 differences between high and mid food). In A. modestus, neither food nor temperature were 226 227 retained in the best models for settlement rate; settlement rates were high (>70%) under all experimental treatments (Fig. 3a). 228

In *S. balanoides*, failure in settlement occurred mainly through reduced cyprid survival (compare figure 2a vs 3a). This is shown by the fact that cyprid survival (i.e. the proportion of settlers calculated over the number of cyprids: Fig. 3c) explains most of the overall larval survival (calculated on the basis of the number of larvae hatched: Fig. 3a). For cyprid survival, both food and temperature were included in the best model (Table 1) in a manner consistent with an independent contribution of temperature and food on survival. In *A. modestus* the proportion ofcyprid larvae settling was high irrespective of temperature (Fig. 3d).

Mortality was higher during the cyprid settlement stage in comparison to the naupliar stages (Table 2). During the cyprid settlement phase for *S. balanoides*, estimated mortality rates were at their highest in the low food treatments (at 9 °C M=0.085 d⁻¹; at 15 °C M=0.157 d⁻¹) when compared to the high and mid food treatments where M<0.058 d⁻¹ (Table 2). In contrast, for *A. modestus* settlement mortality remained low (range 0.017-0.045) and did not vary substantially among food and temperature treatments.

242 *3.2 Developmental time and cyprid size*

For both species, the best models included interactive effects of temperature and food on 243 244 developmental time from hatching to cyprid (Tables 1 and S1). Both species had a shorter developmental time when the larvae experienced the high/mid food rations and/or the higher 245 246 temperature condition (Fig. 4a, b). In S. balanoides, exposure to 15 °C had a disproportionate impact upon duration of larval development under food limited conditions; at 9 °C, development 247 took 18% longer at the low food conditions compared to rates observed at high and mid food 248 levels, but food limitation resulted in an increase of 32% in duration of development at 15 °C (Fig. 249 250 4a). In A. modestus, the effects were proportionally smaller (16-18% longer under low vs highmid food levels). In both species the effect of temperature and food on duration of development 251 252 varied among larvae from different parents (variation among parents was retained in the random 253 structure of the best models: Table S1), but for all parents, larvae took a longer time to develop at the low food level and lower temperature (Fig. 4c, d). Variance components identified that 254 variation in developmental time occurred mainly among parents for most of the experimental 255 treatments, apart from S. balanoides at mid food/15 °C and high food/15 °C where the greatest 256 variation was among replicates. 257

For both species, the best models included interactive effects of food and temperature on cyprid size (Tables 1 and S1). The smallest cyprids were produced at the highest temperature and lowest food (Fig. 5). For *S. balanoides*, increased temperatures led to size reductions of 11-20% depending on food condition, while the low food treatment resulted in a size reduction of 18% at 9 °C and 24% at 15 °C as compared to those reared at high and mid food. For *A. modestus* cyprids, the effect of temperature was small (size reduction in the range 4%-10%); however, cyprids at the low food treatment were 18% smaller at 15 °C and 21% smaller at 18 °C than at high and mid food
for the same temperature. Parental variation was more important for S. *balanoides* than for *A*. *modestus* (Fig. 5c, d, Fig. S2).

267

268 4. Discussion

269 We found that responses of larvae to experimental conditions were consistent with the original 270 hypotheses that a decrease in food abundance would have important consequences for the settlement success of S. balanoides but not for A. modestus. Larval food limitation did not affect 271 272 the success of naupliar development into the cyprid stage, but led to a failure in cyprid settlement in S. balanoides. Under the mid and high food levels, survival compared well with data from 273 274 previous studies for larvae reared under ad libitum food (e.g. Tighe-Ford, 1970; Harms, 1984: percentage survival of >80% for S. balanoides was in the range of 6-18 °C and >70% for A. 275 *modestus* in the range 12-20 °C). 276

Our findings, integrated with information on fertilization, embryogenesis and recruitment, 277 contribute to achieve a better understanding of how increased temperatures can drive population 278 persistence of S. balanoides over the southern part of its distribution range. Because of the 279 temperatures used, the ecological relevance of our analysis is restricted to the southern distribution 280 limit of S. balanoides in Europe (SW Great Britain, N. France, Spain), but not the northern 281 distribution range (including e.g. Spitzbergen: Barnes, 1957b), nor the Atlantic coast of N. 282 America where larvae experience much lower temperatures than those tested here (Pineda et al., 283 284 2005). The same holds for A. modestus that in Europe reaches the western Mediterranean (Galil, 2009) where larvae are likely to experience temperatures >18 °C. Previous studies have indicated 285 286 that temperature effects on fertilization and embryogenesis of S. balanoides can cause changes in distribution range and failures in recruitment (Rognstad et al., 2014; Crickenberger and Wethey, 287 288 2018). Overall, evidence suggests that the mechanism driving failure in recruitment appears to 289 vary along the southern range of the distribution, with reproductive failures occurring in Spain and 290 France, and mismatches between larvae and phytoplankton occurring further north, in S. England (Crickenberger and Wethey, 2018). We found that at temperatures similar to those of the southern 291 292 limit of distribution of S. balanoides, or in a scenario of warming, food limitation reduces 293 settlement success through delayed effects on the proportion of cyprids successfully settling rather 294 than an effect on nauplius survival. Such a delayed effect appears to be similar to that reported in 295 other barnacles (Thiyagarajan et al., 2002; Tremblay et al., 2008). In S. balanoides, low settlement 296 success may reflect reduced levels of nutritional reserves (Jarret et al., 2003) rather than costs 297 associated with settling with a smaller body size. A large amount of such reserves are consumed in the processes of settlement and metamorphosis (Lucas et al., 1979). By contrast, for the tested 298 temperatures, no such reduction in settlement success was detected in A. modestus. We do not 299 300 know how A. modestus larvae would respond to food limitation if larvae were to experience temperatures >18 °C; the highest recorded survival rates are achieved at 24-25 °C (Barker 1972, 301 Harms, 1987) but no information is available on larvae performance under food limitation above 302 18 °C. 303

When responses in cyprid body size are integrated with those of naupliar developmental time 304 305 (summarised in Fig. 6) we see important pointers towards potential fitness costs of developing 306 under combinations of high temperature and food limitation. Those costs would be manifested as effects of larval experience on body size of juveniles and on the timing of metamorphosis, which 307 308 subsequently may drive post-settlement or post-metamorphic survival (Thiyagarajan et al., 2005; Emlet and Sadro, 2006; Torres et al., 2016). We interpret those changes as driven by phenotypic 309 plasticity because naupliar survival was high across treatments. In cases where phenotypic 310 responses occur with a concomitant mortality, the observed phenotypic changes may occur due to 311 312 phenotypic selection (Hechtel and Juliano, 1997). For instance, if food limitation impacts 313 negatively only those individuals characterized by high growth rate, then the survivors would be over-represented by individuals characterized by low growth rates; hence, the average body size 314 would be biased towards lower values. In our case however, phenotypic selection should play a 315 minor role in driving trait responses; in addition, responses were consistent among larvae obtained 316 from different parents. Low food levels led to correlated responses of increasing developmental 317 time and reducing body size, consistent with general predictions of models of metamorphosis 318 319 (Hentschel, 1999; Hentschel and Emlet, 2000; West and Costlow, 1987). In S. balanoides, maximum body size was contingent on temperature, consistent with the temperature-size rule 320 (Atkinson, 1994; Zuo et al., 2012). A. modestus was able to maintain similar body sizes across the 321 range of temperatures experienced (15-18 °C) despite changes in the developmental time; 322 reductions in body size may however occur above 18 °C as suggested by the reduction of energy 323 content in larvae reared at 24 °C (Harms, 1987). In the field, one would expect consequences 324

associated with arriving late, such as limited access to available substratum, and costs associated
with reduced body size such as reduced growth and survival (Jarret et al., 2003; Torres et al.,
2016).

328 An important result was that responses were consistent across larvae obtained from different parents. We observed some variation among parents in the responses for *S. balanoides* which may 329 be related to embryonic experience (Lucas and Crisp, 1987), but larval performance was 330 331 consistently reduced under food limitation. Hence, in both species, but particularly in A. modestus, 332 there were little effects of the parental environment or genetic variation on larval performance. 333 The response of S. balanoides larvae to high temperature and low food levels was found both in our preliminary and definitive experiments (Figs. S1 and 3), based on larvae obtained from parents 334 collected from two different intertidal shores and in different years. Hence, our experiments do not 335 336 provide evidence for strong variation for tolerance to food limitation at 15 °C, at least at the scale 337 of our local population. Some variation for tolerance to food limitation and increased temperature, 338 in particular in S. balanoides, would have pointed to potential room for an evolutionary response 339 to climate change (Appelbaum et al., 2014). In other cases, important intraspecific variation is found within a local population (Carter et al., 2013; Spitzner et al., 2019). Perhaps populations 340 located towards the southern limit of distribution show increased tolerance to food limitation under 341 increased temperature. 342

343 **5.** Conclusions

Overall, our study highlights the stress effects of increased temperature and food limitation on 344 larval performance of S. balanoides, which may translate to settlement failure in the field. Stress 345 responses are manifested as a delayed effect, at the time when cyprids attempt to settle, rather than 346 as an effect on naupliar survival or metamorphosis to the cyprid stage. Given that the effect of food 347 348 limitation on larval performance can increase with temperature (Torres and Giménez, 2020), it remains to be determined if the sensitivity of S. balanoides larvae to low food levels decreases 349 350 towards lower temperatures (3-6 °C) experienced in the northern sector of the distribution range. 351 In larvae of A. modestus, such effects do not occur although larvae of the local population are 352 expected to develop at 14-16 °C.

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354 **Conflict of interest:** All authors declare no conflict of interest

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TABLES

Table 1. Summary of best models (fixed structure) explaining the effect of temperature and food
limitation on survival, developmental time and body size of larvae and metamorphs of barnacles *Semibalanus balanoides* and *Austrominius modestus*. Response variables Abbreviations: Factors:
F: food; T: temperature; interactions are abbreviated as F:T. For details on model selection are
given in Tables S1-S2.

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Response variable	S. balanoides	A. modestus	Suppl Tables
Survival: Hatching to Cyprid (log)	None	F+T	S 1
Dev.time: Hatching to Cyprid	F:T	F:T	S 1
Dev.time: Hatching to Cyprid (log)	F:T	F+T	S 1
Settlement success: from hatching (log)	F+T	None	S2
Settlement success: from cyprid (log)	F+T	F	S 2
Size of cyprids	F:T	F:T	S 1

	Hatching to cyprid		Cyprid to settlement	
S. balanoides	T=9 °C	T= 15 °C	T=9°C	T= 15 °C
Food: Low	0.00465	0.01334	0.08574	0.15727
Food: Mid	0.00406	0.00972	0.03433	0.05136
Food: High	0.00131	0.00767	0.03175	0.05753
A. modestus	T= 15 °C	T=18 °C	T= 15 °C	T= 18 °C
Food: Low	0.00826	0.00526	0.02832	0.02735
Food: Mid	0.00426	0.00363	0.03000	0.03981
Food: High	0.00476	0.00364	0.01712	0.04510

Table 2 Estimated instantaneous mortality rates (average in d⁻¹) of larvae from hatching to cyprid
and cyprid to post-settlement in response to food and temperature.

566 FIGURE CAPTIONS Figure 1. Experimental design used to evaluate the effect of temperature and food limitation on 567 larval development of barnacles S. balanoides and A. modestus. Larvae of each species were 568 reared in separate experiments differing in the temperature treatment. For each species, larvae 569 570 from each parent were also reared separately. Figure. 2 Percentage survival to the cyprid stage for (a) S. balanoides and (b) A. modestus; and 571 572 percentage survival to the cyprid stage for each parent (c) S. balanoides and (d) A. modestus at different food treatments and temperatures. Error bars: (a) and (b) SD based on replicate parents. 573 574 (c) and (d):SD. Letters "ns" indicate non-significant differences among treatments. 575 Figure. 3 Effects of food density and temperature on settlement rates on S. balanoides and A. 576 modestus. (a) and (b) show cyprid settlement as percentage of initial number of larvae; (c) and (d= show it as percentage of developed cyprids. Error bars; SD. Lower case letters indicate significant 577 578 differences (p < 0.005) among food treatments; ns indicates non-significant differences among

- 579 treatments.
- Figure. 4 Effects of food density and temperature for *Semibalanus balanoides* and *Austrominius modestus* Average duration of development to the cyprid stage for (a) *S. balanoides* and (b) *A. modestus;* and average duration of development to the cyprid stage for each parent (c) *S. balanoides* and (d) *A. modestus* at different food treatments and temperatures. Error bars: (a) and (b) SD based on replicate parents. (c) and (d):SD. Different lower case letters show significant differences (p<0.05) between food treatments for each species.
- Figure 5. Cyprid area for (a) *S. balanoides* and (b) *A. modestus;* and cyprid area of larvae from
 each parent (c) *S. balanoides* and (d) *A. modestus* at different food treatments and temperatures.
 Error bars: (a) and (b) SD based on replicate parents. (c) and (d): SD. Different lower case letters
 show significant differences (p<0.05) among food treatments for each species.
- Figure 6. Integrated responses of body size and developmental time under different temperatures
 (9°C, 15°C, 18°C), and food density (low, mid, high). Error bars represent SD among larvae
 produced by different parents (n=4).
- 593



Figure 1

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Figure 2



Figure 3



Figure 4



Figure 5

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