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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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Abstract:	<p>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 <i>Semibalanus balanoides</i> and its exotic competitor, the barnacle <i>Austrominius modestus</i>. Larvae were reared at similar food levels but at temperature ranges which varied among species reflecting their different phenology and tolerances. For <i>S. balanoides</i> (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 <i>A. modestus</i> (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 (<i>Skeletonema costatum</i>) at three food levels. We found stress effects of food limitation on larval performance of <i>S. balanoides</i>. 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 <i>S. balanoides</i> 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 exotic <i>A. modestus</i>. Both species delayed development in response to low food levels while <i>S. balanoides</i> 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 metamorphosis to the cyprid stage. Response 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 increases, settlement success of <i>S. balanoides</i> larvae (but not that of its competitor <i>A. modestus</i>) will become more sensitive to conditions of food limitation, imposed for instance by phenological mismatches with periods of phytoplankton peak.</p>
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Opposed Reviewers:	
Response to Reviewers:	

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,

A handwritten signature in blue ink, appearing to read 'Luis Giménez', is positioned below the word 'Sincerely,'.

Dr. Luis Giménez

Biologische Anstalt Helgoland, Alfred Wegener Institute,

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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

3
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27 **Abstract**

28 A critical question in marine ecology is understanding how organisms will cope with
29 environmental conditions under climate change. Increasing temperatures not only have a direct
30 effect on marine organisms but may also lead to food limitation through for example trophic
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
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35 *modestus*. Larvae were reared at similar food levels but at temperature ranges which varied among
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39 typical summer temperature for this geographic range of 15°C was used with a raised temperature
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52 increases, settlement success of *S. balanoides* larvae (but not that of its competitor *A. modestus*)
53 will become more sensitive to conditions of food limitation, imposed for instance by phenological
54 mismatches with periods of phytoplankton peak.

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

57 **1. Introduction**

58 A key current question in ecology concerns the action of multiple climate driven variables on
59 organisms, populations and communities. Climate change involves a modification of a large
60 number of environmental variables which can act simultaneously, potentially altering performance
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
63 factors are handled by organisms (Spitzner et al., 2019). For example, in the marine habitat, larval
64 stages of crustaceans and fish are thought to experience a food limited environment (Barnes, 1956;
65 Olson and Olson, 1989; Gotceitas et al., 1996; Gimenez and Anger, 2005; Le Pape and
66 Bonhommeau, 2015). In seasonal habitats, increases in temperature can be accompanied by
67 reductions in food availability because of phenological mismatches, between periods of larval
68 development and that of food production (Edwards and Richardson, 2004). In addition, and
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 Ortégón 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;
75 Poloczanska et al., 2013), which can produce important modifications in natural communities if
76 they become invasive (Simberloff, 2009; Gurevitch et al., 2011). In regions where warming is
77 accompanied by the arrival of exotic species, the overall result is that native species are faced with
78 harsher habitats but also need to invest extra resources in competing with exotic species. An
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
82 competitor fare in the light of warming.

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

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

110 **2. Materials and Methods**

111 *2.1 Experimental design*

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

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

137 2.2. Collection of animals and culturing procedures

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

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

150 Diatoms *Skeletonema costatum* (strain: CCAP 1077/5) were provided at appropriate cell densities
151 every 2 days with provision of algae coinciding with the time of water change. Prior to each
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.
155 Larval culture water was changed by pouring the contents of a replicate jar into a small glass bowl
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
161 days until the first appearance of cyprids, after which time, larval cultures were checked daily
162 (with water change and feeding still taking place every 2 days). All cultures ran until larvae had
163 either died or moulted to the final larval cyprid stage. When the cyprid stage was reached, cyprids
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
167 incubator in FSW at the same temperature they were reared under. Ten cyprids from each adult at
168 each treatment were selected at random and photographed using a microscope camera GX-CAM
169 5. Photographs were subsequently analysed using Image J to calculate the length, width and cyprid
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
172 observed. Clean rock scraped from conspecific barnacles were provided as a settlement surface.
173 On day 2, 4, 6 and 8 (after the rock had been added) the cyprid cultures were examined and the
174 water was changed. During examination, numbers of metamorphosed cyprids upon the rocks were
175 counted and any dead cyprids in the jar were removed.

176

177 2.3 Data analysis

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

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

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

198 Estimates of variance components were used to evaluate patterns of variability in the response
199 variables among random factors (shore, parent and replicate) for each experimental treatment. The
200 relative variance components were estimated for each source of variation by using the observed
201 mean squares to estimate terms identified in the expected mean squares (Searle et al. 1992;
202 Underwood, 1997; Graham and Edwards, 2001). Sometimes one or more estimates from the
203 ANOVA method were negative; then, these estimates were set to zero, removed from the model
204 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 comparable
206 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
210 experimental treatments (Fig. 2a, b); hence, effects of food limitation or temperature on survival
211 were weak. For *S. balanoides*, the best models did not include food or temperature as main factors
212 driving survival to the cyprid stage (Table 1). The effect of food and temperature on survival rates
213 of *S. balanoides* varied among individuals hatching from different parents (best model included
214 parent of origin in their random part: supplementary material: Table S1). Most important variation
215 among parents of *S. balanoides* occurred at the high temperature, especially at low food levels
216 (supplementary material: Fig. S2). For *A. modestus*, the best models retained food and temperature
217 but the actual effect was weak, as survival was very high, >90%, under all experimental conditions
218 (Fig. 2b). Post-hoc tests on averages by parent did not detect differences among treatments and
219 variance components indicated low contribution of parents to the total variability for this species
220 (supplementary material: Fig. S2).

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

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

234 independent contribution of temperature and food on survival. In *A. modestus* the proportion of
235 cyprid larvae settling was high irrespective of temperature (Fig. 3d).

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

242 3.2 Developmental time and cyprid size

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

258 For both species, the best models included interactive effects of food and temperature on cyprid
259 size (Tables 1 and S1). The smallest cyprids were produced at the highest temperature and lowest
260 food (Fig. 5). For *S. balanoides*, increased temperatures led to size reductions of 11-20%
261 depending on food condition, while the low food treatment resulted in a size reduction of 18% at
262 9 °C and 24% at 15 °C as compared to those reared at high and mid food. For *A. modestus* cyprids,
263 the effect of temperature was small (size reduction in the range 4%-10%); however, cyprids at the

264 low food treatment were 18% smaller at 15 °C and 21% smaller at 18 °C than at high and mid food
265 for the same temperature. Parental variation was more important for *S. balanoides* than for *A.*
266 *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
271 settlement success of *S. balanoides* but not for *A. modestus*. Larval food limitation did not affect
272 the success of naupliar development into the cyprid stage, but led to a failure in cyprid settlement
273 in *S. balanoides*. Under the mid and high food levels, survival compared well with data from
274 previous studies for larvae reared under ad libitum food (e.g. Tighe-Ford, 1970; Harms, 1984:
275 percentage survival of >80% for *S. balanoides* was in the range of 6-18 °C and >70% for *A.*
276 *modestus* in the range 12-20 °C).

277 Our findings, integrated with information on fertilization, embryogenesis and recruitment,
278 contribute to achieve a better understanding of how increased temperatures can drive population
279 persistence of *S. balanoides* over the southern part of its distribution range. Because of the
280 temperatures used, the ecological relevance of our analysis is restricted to the southern distribution
281 limit of *S. balanoides* in Europe (SW Great Britain, N. France, Spain), but not the northern
282 distribution range (including e.g. Spitzbergen: Barnes, 1957b), nor the Atlantic coast of N.
283 America where larvae experience much lower temperatures than those tested here (Pineda et al.,
284 2005). The same holds for *A. modestus* that in Europe reaches the western Mediterranean (Galil,
285 2009) where larvae are likely to experience temperatures >18 °C. Previous studies have indicated
286 that temperature effects on fertilization and embryogenesis of *S. balanoides* can cause changes in
287 distribution range and failures in recruitment (Rognstad et al., 2014; Crickenberger and Wethey,
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
291 (Crickenberger and Wethey, 2018). We found that at temperatures similar to those of the southern
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
298 in the processes of settlement and metamorphosis (Lucas et al., 1979). By contrast, for the tested
299 temperatures, no such reduction in settlement success was detected in *A. modestus*. We do not
300 know how *A. modestus* larvae would respond to food limitation if larvae were to experience
301 temperatures >18 °C; the highest recorded survival rates are achieved at 24-25 °C (Barker 1972,
302 Harms, 1987) but no information is available on larvae performance under food limitation above
303 18 °C.

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

325 associated with arriving late, such as limited access to available substratum, and costs associated
326 with reduced body size such as reduced growth and survival (Jarret et al., 2003; Torres et al.,
327 2016).

328 An important result was that responses were consistent across larvae obtained from different
329 parents. We observed some variation among parents in the responses for *S. balanoides* which may
330 be related to embryonic experience (Lucas and Crisp, 1987), but larval performance was
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
334 our preliminary and definitive experiments (Figs. S1 and 3), based on larvae obtained from parents
335 collected from two different intertidal shores and in different years. Hence, our experiments do not
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
340 found within a local population (Carter et al., 2013; Spitzner et al., 2019). Perhaps populations
341 located towards the southern limit of distribution show increased tolerance to food limitation under
342 increased temperature.

343 **5. Conclusions**

344 Overall, our study highlights the stress effects of increased temperature and food limitation on
345 larval performance of *S. balanoides*, which may translate to settlement failure in the field. Stress
346 responses are manifested as a delayed effect, at the time when cyprids attempt to settle, rather than
347 as an effect on naupliar survival or metamorphosis to the cyprid stage. Given that the effect of food
348 limitation on larval performance can increase with temperature (Torres and Giménez, 2020), it
349 remains to be determined if the sensitivity of *S. balanoides* larvae to low food levels decreases
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.

353

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.

Response variable	<i>S. balanoides</i>	<i>A. modestus</i>	Suppl Tables
Survival: Hatching to Cyprid (log)	None	F+T	S1
Dev.time: Hatching to Cyprid	F:T	F:T	S1
Dev.time: Hatching to Cyprid (log)	F:T	F+T	S1
Settlement success: from hatching (log)	F+T	None	S2
Settlement success: from cyprid (log)	F+T	F	S2
Size of cyprids	F:T	F:T	S1

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

	Hatching to cyprid		Cyprid to settlement	
<i>S. balanoides</i>	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
<i>A. modestus</i>	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

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FIGURE CAPTIONS

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Figure 1. Experimental design used to evaluate the effect of temperature and food limitation on larval development of barnacles *S. balanoides* and *A. modestus*. Larvae of each species were reared in separate experiments differing in the temperature treatment. For each species, larvae from each parent were also reared separately.

Figure. 2 Percentage survival to the cyprid stage for (a) *S. balanoides* and (b) *A. modestus*; and 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. (c) and (d):SD. Letters “ns” indicate non-significant differences among treatments.

Figure. 3 Effects of food density and temperature on settlement rates on *S. balanoides* and *A. 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 differences ($p < 0.005$) among food treatments; ns indicates non-significant differences among 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).

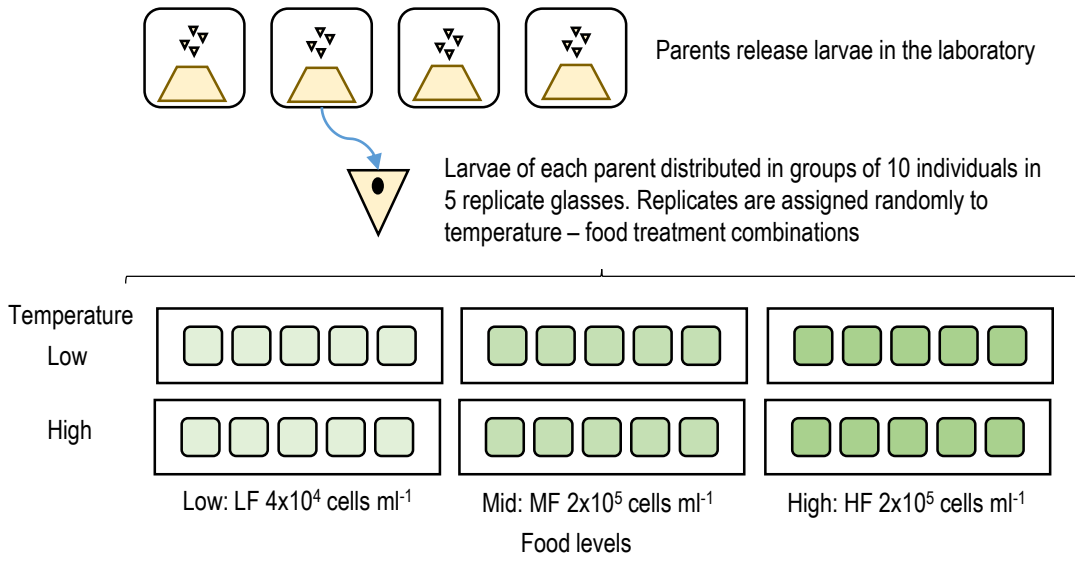


Figure 1

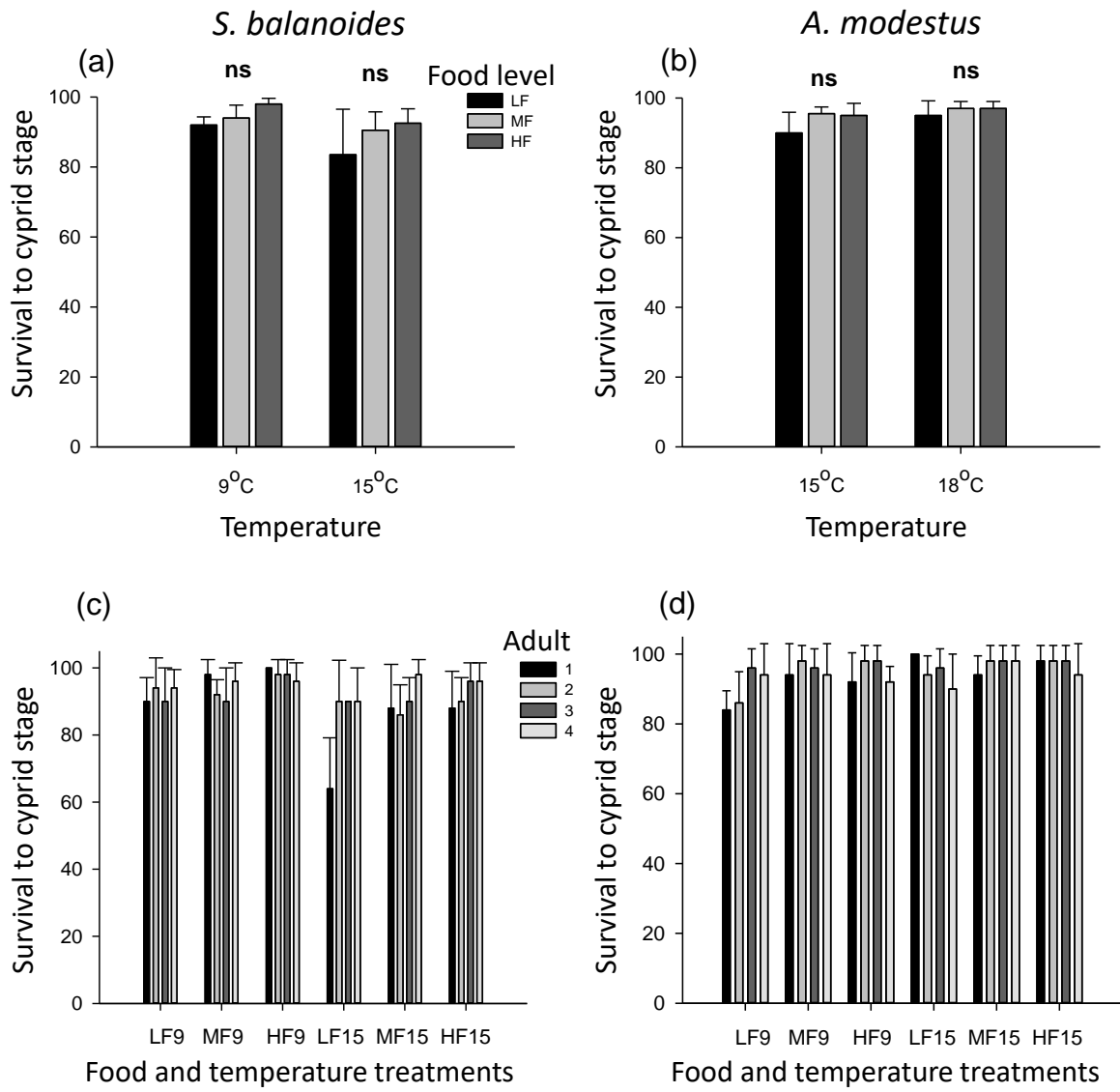


Figure 2

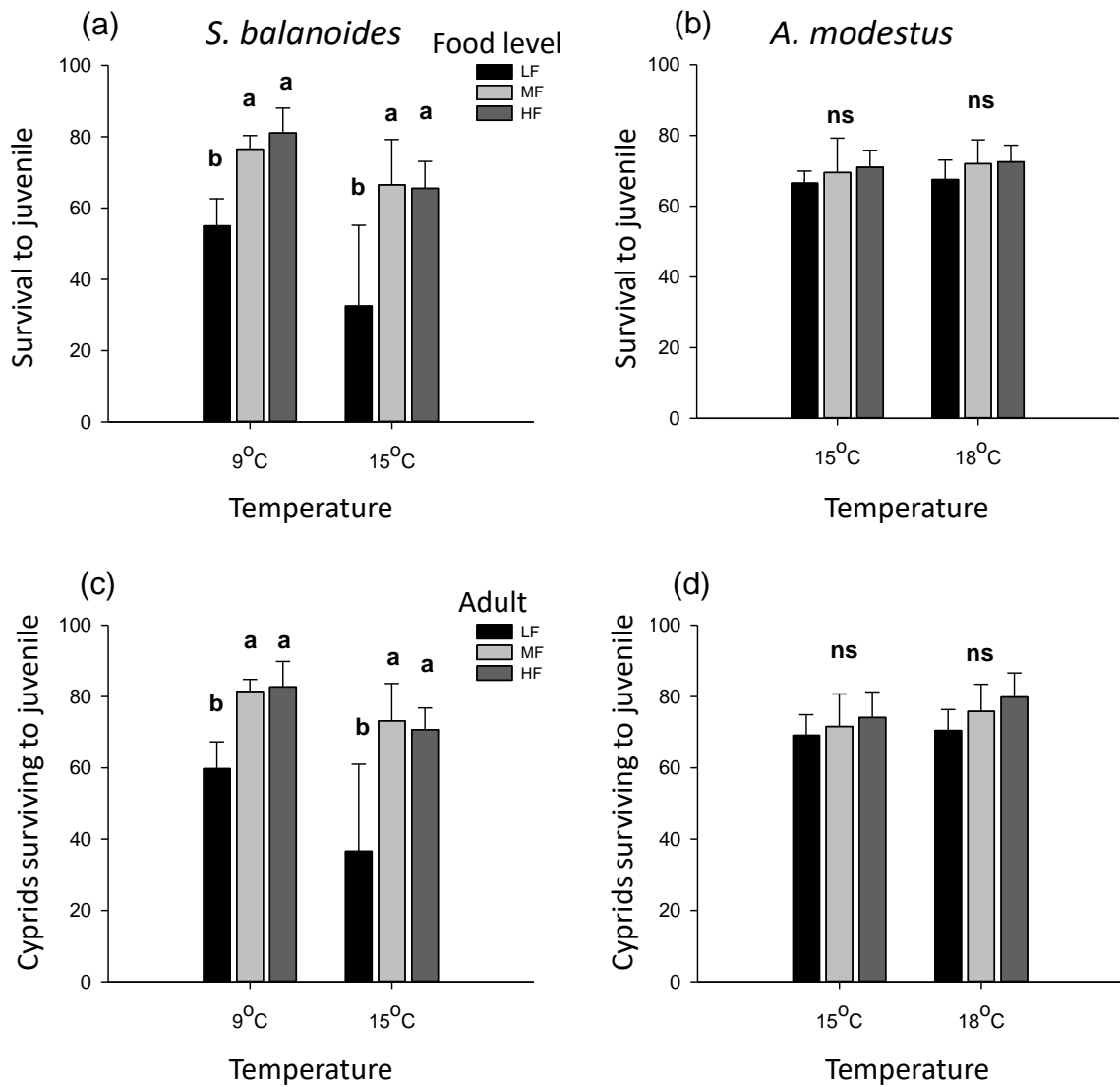


Figure 3

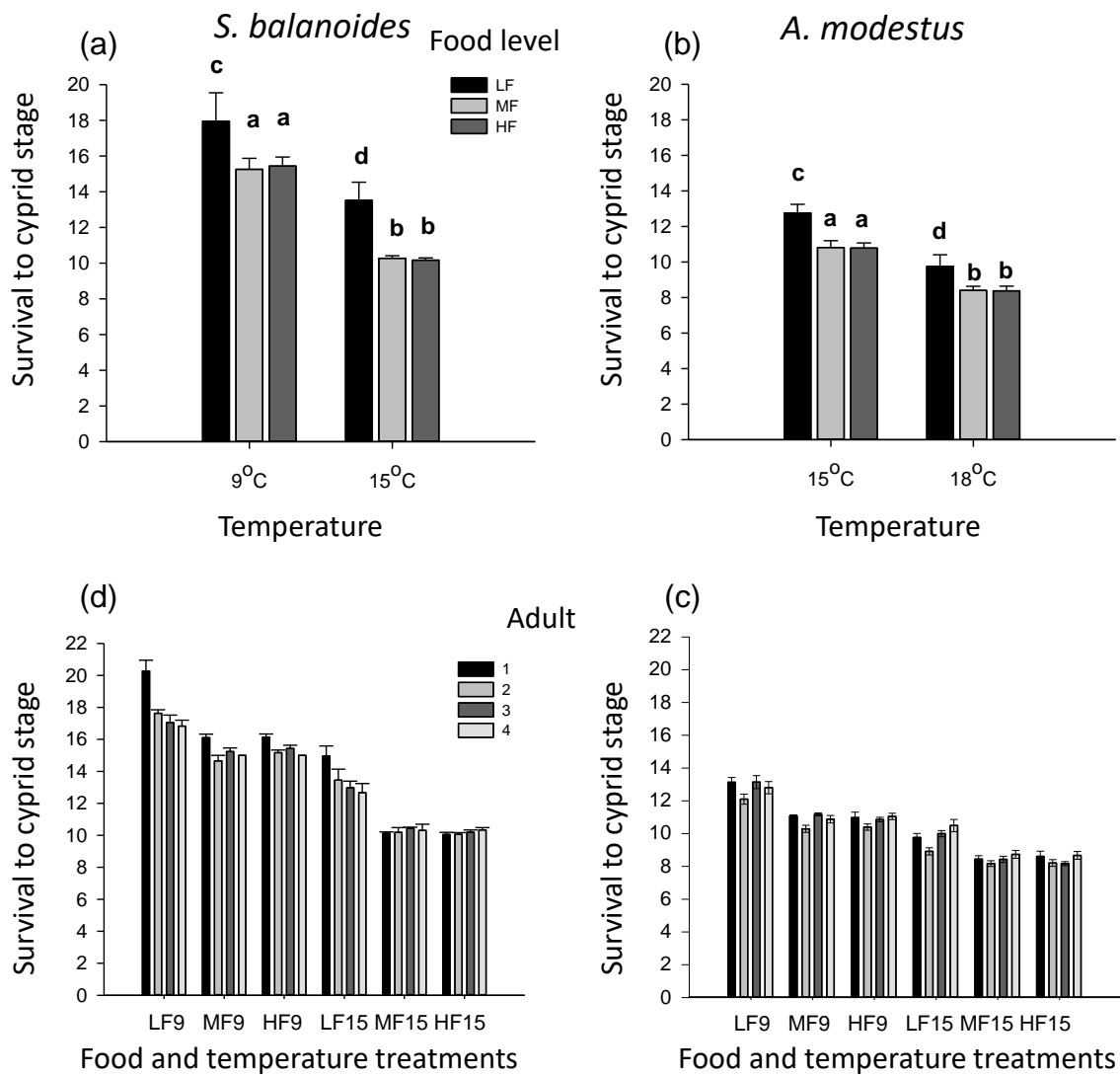


Figure 4

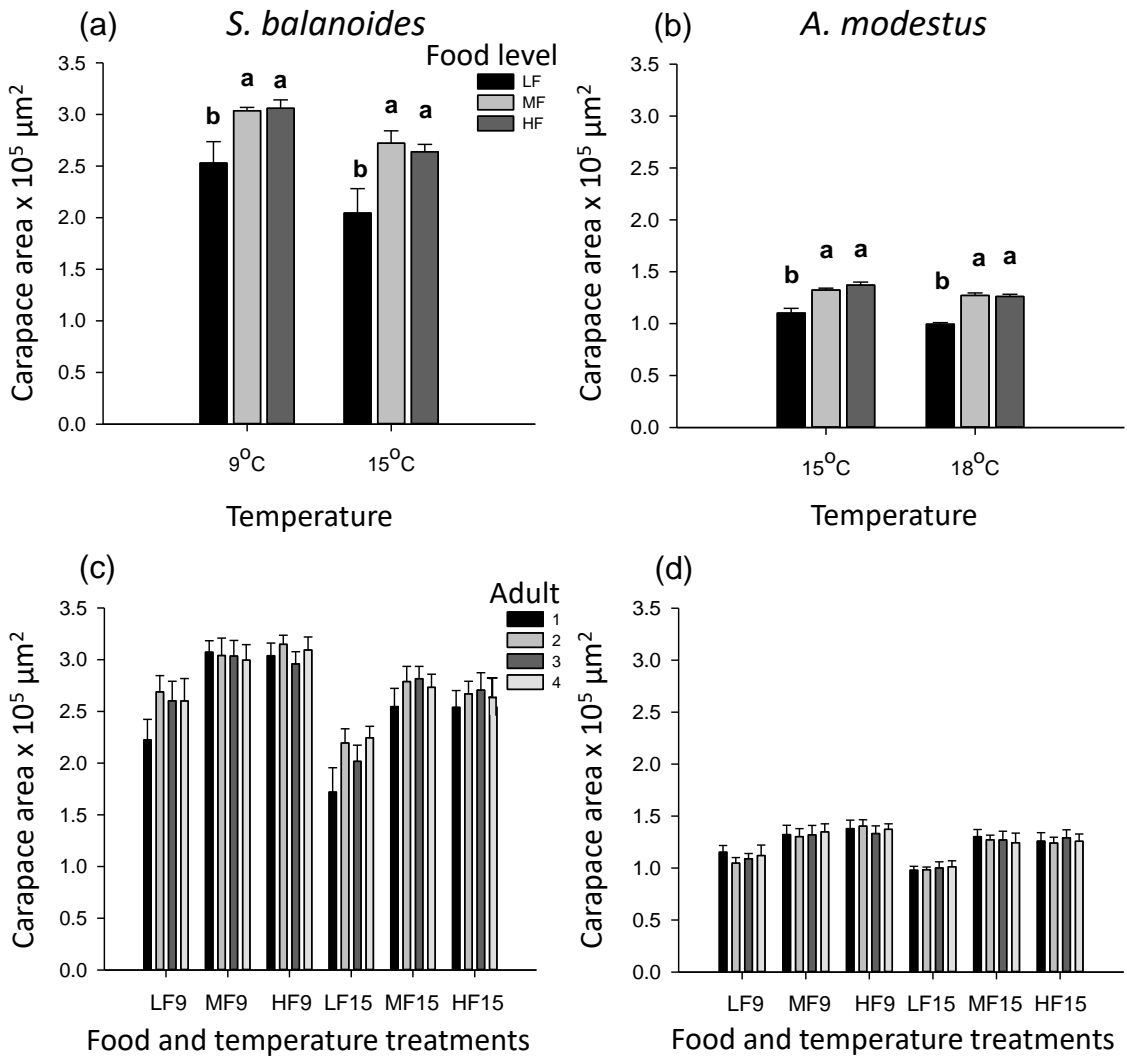
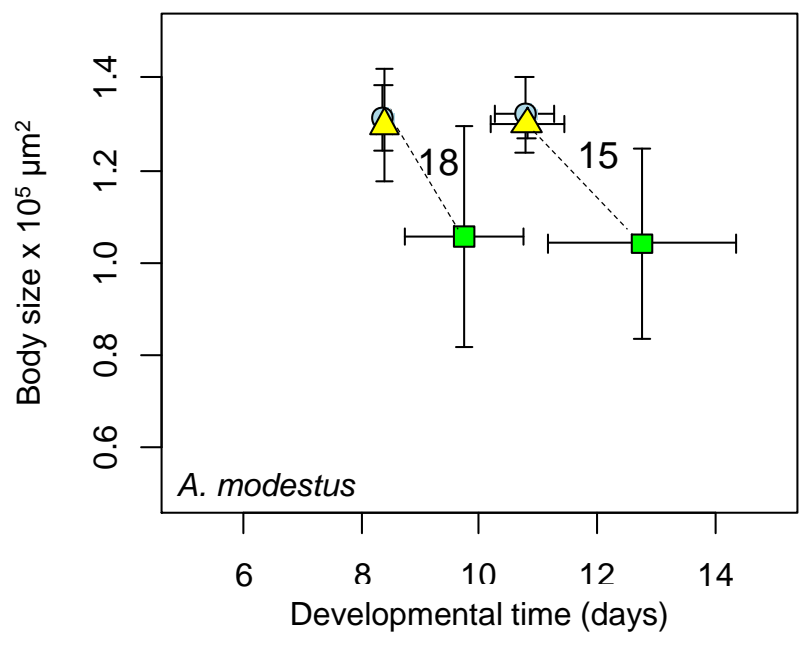
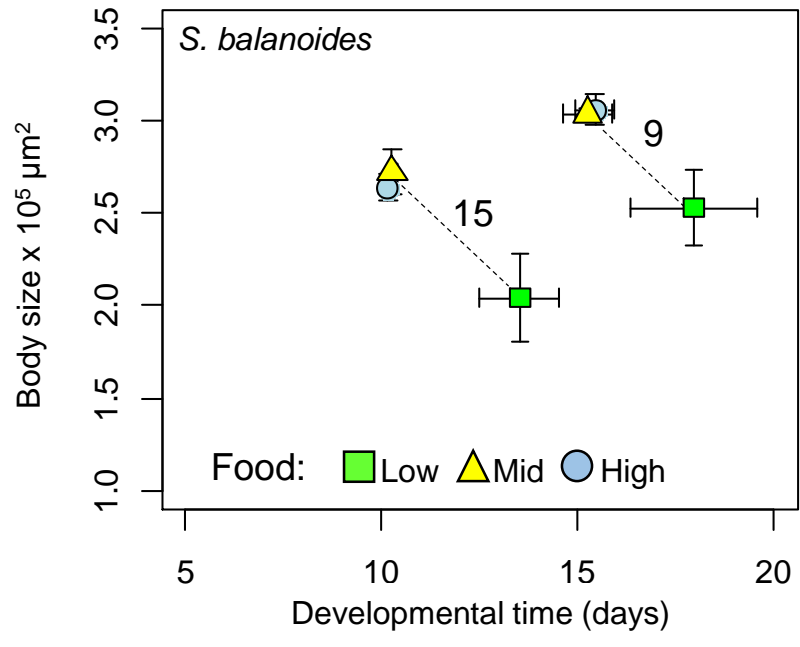


Figure 5





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