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1 **Effects of culture conditions on larval growth and survival of stalked barnacles**
2 **(*Pollicipes pollicipes*)**

3 Sofia C. Franco^{a,b}, Nick Aldred^a, Teresa Cruz^{b,c}, Anthony S. Clare^a

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5 ^aSchool of Marine Science and Technology, Newcastle University, Newcastle upon
6 Tyne, NE1 7RU, United Kingdom

7 ^bDepartamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora,
8 Évora , Portugal

9 ^cMARE – Marine and Environmental Sciences Centre, Laboratório de Ciências do Mar,
10 Universidade de Évora, Portugal

11

12

13 E-mail: sofia.cota-franco@newcastle.ac.uk

14 Phone: +44(0)191 208 6653

15 Fax: +44(0)191 208 7891

16 Address: School of Marine Science and Technology, Newcastle University, Ridley
17 Building, Claremont Rd, Newcastle upon Tyne, NE1 7RU, United Kingdom

18

19 Running title: Larval culture of stalked barnacles (*P. pollicipes*)

20 **Abstract**

21 *Pollicipes pollicipes* (Crustacea: Scalpelliformes) is a highly prized food in Portugal and
22 Spain and consequently a species of considerable interest to aquaculture. Surprisingly,
23 however, larval culture conditions for this barnacle have not been optimised. The
24 present study investigated the effects of temperature, diet, photoperiod and salinity on
25 the growth and survival of *P. pollicipes* larvae. Temperature had a significant effect on
26 specific growth rate (2.6 to 5.9 % total width per day, from 11 to 24 °C), reducing mean
27 development time to the cyprid from 25 days at 11 °C to 10 days at 24 °C, though this
28 was accompanied by a significant increase in mortality to over 90 % above 22 °C. Mid-
29 range temperatures (15 – 20 °C) maximised total survival (19 to 31 %, respectively).
30 Algal diets of *Tetraselmis suecica*, *T. suecica/Skeletonema marinoi* and *S. marinoi*/
31 *Isochrysis galbana* did not affect specific growth rate significantly, but survival (on
32 average 39 % in 15 days) and the proportion of high-quality, healthy cyprids were
33 significantly higher on the latter two diets (11 to 15 % of initial number of larvae).
34 Photoperiod did not significantly affect the survival, although specific growth rate was
35 significantly higher at 24:0 and 16:8 L:D. Salinity (20 – 40 gL⁻¹ range) did not affect
36 growth and survival significantly. The best growth and survival were accomplished
37 using rearing temperatures of 15 – 20 °C, daily feeding with *T. suecica/S. marinoi* or *I.*
38 *galbana/S. marinoi* and a photoperiod of 24:0 L:D.

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40 **Keywords:** larval development; temperature; diet; photoperiod; salinity; aquaculture.

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44 **1. Introduction**

45 *Pollicipes pollicipes* (Gmelin, 1790) is one of the most commercially important
46 barnacle species for human consumption (López *et al.*, 2010), being heavily exploited in
47 Spain and Portugal (Borja *et al.* 2006, Sousa *et al.* 2013). In recent years, as concerns
48 have grown over stock management, interest has risen in the aquaculture of this species.
49 The few published investigations into culture conditions for *P. pollicipes* (e.g.
50 Goldberg, 1984; Coelho, 1990, 1991; Molares *et al.*, 1994a, 1994b; Candeias, 2005;
51 Cribeiro, 2007; Franco *et al.*, 2015) have often identified larval production and
52 settlement as the main bottlenecks to production. Hatchery production is essential to
53 ensure a regulated supply of larvae throughout the year with controlled timing and yield
54 (Franco *et al.*, 2015), as well as to guarantee high larval quality. Furthermore, larval
55 collection from the wild provides an unreliable solution for aquaculture, possibly
56 presenting conservation issues and conflicts with stock management programmes.
57 Larval culture and settlement of various barnacle species have long been investigated
58 (e.g. Knight-Jones & Stevenson, 1950; Knight-Jones, 1953; Keough & Downes, 1982;
59 Brown & Roughgarden, 1985; Gabbott & Larman, 1987; Maki *et al.*, 1988) and
60 although protocols for species such as *Elminius modestus* (*Austrominius modestus*),
61 *Balanus improvisus* (*Amphibalanus improvisus*) and *Balanus amphitrite* (*Amphibalanus*
62 *amphitrite*) are well established (after Moyses, 1963; Tighe-Ford *et al.*, 1970), protocols
63 for others such as *P. pollicipes* are in their infancy.

64 Hatchery production of *P. pollicipes* larvae encompasses two key phases: the six stages
65 of naupliar development to the cyprid and cyprid settlement, wherein a suitable surface
66 is located on which to attach permanently. Gametogenesis and larval development of *P.*
67 *pollicipes* have previously been described (Coelho, 1990; Molares *et al.*, 1994a, 1994b)
68 and nauplii have been cultured to the cyprid in several studies despite difficulties in

69 obtaining high survival to this stage (Coelho, 1990; Molaes, 1994; Molaes *et al.*,
70 1994b). Nevertheless, knowledge of the effects of environmental factors and feeding on
71 larval growth and survival of *P. pollicipes* remains poor. In fact, these essential steps are
72 very rarely elaborated upon (e.g. Molaes, *et al.*, 1994b), with most of the available
73 information coming from grey literature sources (e.g. Coelho, 1990; Molaes *et al.*,
74 2002; Candeias, 2005). Studies on the remaining Pollicipedidae, including *Capitulum*
75 *mitella* (Qiu *et al.*, 1994a, 1994b; Lin *et al.*, 1994, 2002; Rao *et al.*, 2010; Zhang *et al.*,
76 2009) and *Pollicipes polymerus* (Lewis, 1975) are also scarce. In the absence of an
77 integrated investigation of optimal larviculture for *P. pollicipes*, the preliminary works
78 of several authors on larval production systems (Molaes, *et al.*, 1994b; Molaes *et al.*,
79 2002), rearing diets (Coelho, 1990, Candeias, 2005) and the effects of temperature and
80 culture density (Coelho, 1990) can provide a basis from which to build. Coelho's (1990)
81 preliminary experiments compared rearing at 15 and 22 °C and showed that nauplii took
82 longer to develop to stage VI at the lower temperature. The same study also suggested
83 that diets of *Tetraselmis suecica* and *Isochrysis galbana* were required to ensure
84 successful development to nauplius VI, although no data are presented. Candeias
85 (2005), who conducted the most detailed study focusing on larval feeding behaviour,
86 reported that satiation slows with development, varying with algal density and energetic
87 value, and gave an indication of preferred diets, such as *I. galbana* and *Skeletonema*
88 *costatum*. It was further suggested that nutritional requirements for early and later stages
89 might differ as the larvae develop. Besides the studies developed by the previous
90 authors on diet and temperature, no studies have investigated the effect of photoperiod
91 and salinity on larval development and survival, with the requirements for larviculture
92 of *P. pollicipes* remaining a research gap.

93 The present study aimed to establish the optimal culture conditions of temperature, diet,
94 photoperiod and salinity for rearing *P. pollicipes* larvae to the cyprid stage. It is
95 hypothesised that there is a range of environmental and dietary conditions that optimise
96 culture performance, relating to natural values experienced in the wild during the
97 reproductive season. Larvae of *P. pollicipes* were reared at a range of natural
98 temperatures (11 to 24 °C), with different monodiets and mixed diets (of *I. galbana*, *T.*
99 *suecica* and *Skeletonema marinoi*), short to long-day photoperiod and a broad salinity
100 range (20 to 40 gL⁻¹) and their growth, survival and quality were followed to the cyprid
101 stage.

102 **2. Materials and Methods**

103 2.1 Collection of broodstock and egg lamellae

104 Clusters of stalked barnacles (*P. pollicipes*) were collected from the south-west coast of
105 Portugal (Cabo Sardão, Portugal, 37°36'24.70", -8°49'2.00") and transported to the
106 rearing facilities at the School of Marine Science of Technology (Newcastle University,
107 UK) within 24h of collection. Barnacles were collected from the same area multiple
108 times during the breeding cycle (July to September) in consecutive years (2011 to
109 2013). Mature egg lamellae (n ≥ 40) were immediately dissected from adult barnacles (n
110 ≥ 60; ≥ 12.5 mm rostro-carinal distance, RC), separated and cut into pieces to assist
111 naupliar release. Healthy nauplii were separated from dead nauplii, unhatched embryos
112 and lamella membranes, by attraction to a point light source and then transferred to
113 culture.

114 2.2 Culture conditions

115 The newly hatched nauplii were sampled and counted to determine larval numbers and
116 divided into experimental groups, each with three independent replicates per treatment.

117 Each replicate culture consisted of a 500-ml conical aquarium (JBL Artemio ®) of 0.22
118 μm filtered natural seawater (NSW), provided with weak bottom aeration. The effect of
119 stocking density was not tested and therefore initial stock densities were kept
120 intentionally low (5 larvae ml^{-1} ; approximately 2500 larvae per aquarium). As it was
121 difficult to acquire sufficient numbers of larvae from the same batch to run all
122 experiments simultaneously or allow for a multifactorial design, experiments were run
123 separately with distinct batches of larvae. Overlap in the conditions tested allowed for
124 standardisation between experiments, although each experiment tested a different
125 environmental factor. Four experiments were conducted, testing temperature
126 (experiment 1), diet (experiment 2), photoperiod (experiment 3) and salinity
127 (experiment 4). Standard culture conditions included a temperature of 20 ± 1 °C (except
128 in experiment 1), light intensity of 2187 ± 102 lux, photoperiod of 16:8 L:D (except in
129 experiment 3), salinity of 33 ± 1 gL^{-1} (except in experiment 4) and antibiotics (0.0232 g
130 l^{-1} penicillin G and 0.0369 g l^{-1} streptomycin). Larvae were fed every 2 days with a
131 mixed diet of *T. suecica* and *I. galbana* (100 000 cells mL^{-1} 1:1) (except in experiment
132 2) after seawater changes.

133 2.3 Experimental design

134 Experiment 1 tested the effect of temperature (11, 15, 20, 22 and 24 °C) on larval
135 growth and survival. This experiment was divided in two, due to limited larval numbers
136 at any one time, with larvae collected from two distinct batches of adults. The first part
137 tested temperatures of 11 ± 1 °C, 15 ± 1 °C and 20 ± 1 °C, while the second part tested
138 temperatures of 20 ± 1 °C, 22 ± 1 °C and 24 ± 1 °C. Each treatment was kept in separate
139 temperature-controlled incubators (LabHeat© and LabCold© RLCH0400 Incubator
140 Units, UK). Cultures were sampled daily (as described in section 2.4) until over 50 % of

141 larvae had reached the cyprid stage in all replicates, at which point the treatment was
142 terminated.

143 Experiment 2 tested the effect of diet on larval growth and survival by using various
144 monodiets and mixed diets of *I. galbana*, *T. suecica* and *S. marinoi*. Diets were as
145 follows: (a) *I. galbana*; (b) *T. suecica*; (c) *S. marinoi*; (d) *I. galbana* and *S. marinoi*; (e)
146 *I. galbana* and *T. suecica*; and (f) *T. suecica* and *S. marinoi*. Cultures were provided
147 with 100 000 cells ml⁻¹, which were in a 1:1 ratio for mixed diets (50 000 cells ml⁻¹ of
148 each food item). Algae were cultured in 10-L carboys in autoclaved NSW, 20 ± 1 °C, 37
149 ± 1 gL⁻¹, 16:8 L:D photoperiod, 2022 ± 58 lux (RS-01 Light Meter©, UK), grown with
150 F/2 medium (Guillard & Ryther, 1962; Guillard, 1975).

151 Experiment 3 tested the effect of photoperiod on larval growth and survival. The larvae
152 were reared under the following photoperiods: (a) 24:0 L:D; (b) 16:8 L:D; (c) 8:16 L:D;
153 and (d) 0:24 L:D. Treatments were kept in temperature- and photoperiod-controlled
154 incubators (LabHeat© RLCH0400 Incubator Unit, UK), with light intensities of 2245 ±
155 256 lux (lamps Osram Fluora, L 36W/77, UK).

156 Experiment 4 examined the effects of salinity (20, 30 and 40 gL⁻¹) on larval growth and
157 survival. Salinity was checked daily with a refractometer (Hand Held Refractometer
158 B+S©) and adjusted accordingly, to account for variations caused by feeding and water
159 evaporation.

160 2.4 Data collection and analysis

161 Cultures were sampled (n=3) every other day, before feeding. A given volume was
162 filtered (80 µm mesh) from each culture and the larvae were preserved with Lugol's
163 iodine solution, until analysis. Due to limiting larval numbers and in order to minimise

164 sampling impact, the volume to be sampled was calculated at every sampling event and
165 adjusted for the changing culture density to ensure that a minimum of 30 nauplii per
166 sample were taken from each replicate. Samples were imaged using a stereo microscope
167 and the total number of larvae counted. Each larva was classified according to
168 developmental stage (following Molares, 1994; Kugele & Yule, 1996), photographed
169 (Olympus© E-410) and measured using the software Image J© (v1.49).

170 Total width for nauplii (TW) and carapace width for cyprids (CW) were used to
171 estimate the larval greatest width (LGW). Stage progression index (SPI, #), calculated
172 using the total number of larvae per each developmental stage at any one time, was used
173 as a measure of larval development through the culture period. This was calculated by
174 $[SPI = (\sum_{i=1}^n (n_i \times s_i)) / n]$, where n is total number of larvae, n_i is number of individuals at
175 development stage i , and s_i is development stage i (with $i=1, 2, 3, 4, 5, 6$ and 7 ,
176 respectively for nauplii I, II, III, IV, V, VI and cyprids) at a given time. Larval specific
177 growth rate (SGR, %TW d^{-1}), which factored nauplius TW and total time of
178 development until nauplius VI dominance, was used to assess naupliar growth. This was
179 calculated as $[SGR = (\ln((avTW_{t_f}) / avTW_{t_i}) / (t_f - t_i) \times 100)]$, where SGR is specific growth
180 rate as a percentage of total width increase per day, $avTW$ is the average naupliar total
181 width per replicate, t_i is initial time or day 0 when 50 % of larvae are nauplius I and t_f is
182 final time when 50 % of larvae reached nauplius VI. Median culture development time
183 from nauplius I to the cyprid (MDT, days or d) was calculated at the time when
184 sampling ended by $[MDT = t_f - t_i]$, where t_i is initial time or day 0 when 50 % of larvae
185 are at nauplius I and t_f is final time or day when ≥ 50 % larvae are cyprids.

186 Each culture was followed until the majority comprised cyprids (≥ 50 %), at which time
187 the experiments were terminated by filtering the culture through a 60 μm mesh to

188 collect all larvae. The larvae were then counted, measured and classified according to
189 stage. These final samples were used to determine the total number of cyprids produced
190 and to visually estimate the proportion of high-quality larvae. For the visual estimation,
191 all larvae (nauplii and cyprids) were compared for activity (static or active), appearance
192 of appendages (damaged/protruded or normal) and fouling of the larvae (fouled or
193 unfouled), with cyprids further observed for the presence of lipid droplets (absent or
194 present). Larvae were classified as high-quality larvae (H-Q larvae, %) if they satisfied
195 the majority of the above-mentioned criteria for healthy larvae (i.e. larvae that were
196 active, had healthy appendages, were unfouled and had visible lipidic droplets when at
197 the cyprid stage). The percentage of high-quality larvae H-Q larvae (HQ_l , %) was
198 calculated by [$HQ_l = nHQ_l/n_f$], where nHQ_l is the number of high-quality larvae collected
199 at the end of the experiment and n_f is the total number of collected larvae. The
200 percentage of cyprids (C, %) was calculated by [$C = nC/n_f$], where nC is the number of
201 cyprids collected at the end of the experiment and n_f is the total number of larvae
202 collected. Both the percentage of H-Q larvae and cyprids were calculated in relation to
203 the total number of larvae collected in each replicate at the end of the experiment. The
204 total percentage of high-quality cyprids (H-Q cyprids, %) was calculated with regard to
205 the number of larvae initially stocked per replicate and the number of high-quality
206 cyprids counted at the end of the experiment, as [$HQ_c = nHQ_c/n_i$], where nHQ_c is the
207 number of high-quality cyprids collected at the end of the experiment and n_i is the total
208 number of larvae initially stocked at day 0.

209 Percentage survival (tS, %) was calculated at the end of the experiment using [$S = n_f/n_i$],
210 where n_f is the total number of larvae at the time point where sampling was terminated
211 and n_i is the total number of larvae initially stocked at day 0. The survival rate (dS, % d⁻¹)
212 and mortality rate (dM, % d⁻¹) were calculated on a daily basis. Although sampling

213 effort was adjusted to minimise impact, sampling was nevertheless extractive. The
214 removal of larvae was not accounted for in the calculations (values presented are not-
215 corrected), but the maximum impact of sampling was estimated, considering the
216 numbers of extracted larvae per replicate, and implications for survival will be
217 discussed later.

218 All variables were calculated per replicate and results were analysed using Statistica ®
219 (v.7.0.61.0, StatSoft Inc.) at a significance level of 0.05. Analyses were carried out to
220 determine homogeneity of variance (Levene's test) and normality (Kolmogorov-
221 Smirnov test), while significant differences were detected using one-way ANOVA or
222 ANCOVA with time as a covariate where appropriate. Post-hoc investigation used
223 Tukey's HSD test when relevant. Data in percentage format were arcsine transformed
224 prior to analysis. All results are presented as mean \pm standard error, unless stated
225 otherwise.

226 **3. Results**

227 3.1 Temperature

228 Specific growth rate (SGR) was significantly influenced by both the low (ANOVA,
229 $F=57.50$, $p<0.01$) and high (ANOVA, $F=48.47$, $p<0.01$) temperatures. When both
230 experiments were analysed concurrently, the data for the overlapping temperature (20
231 °C) did not differ significantly between experiments (ANOVA, $F=57.5$, $p<0.01$; Tukey
232 Test $p=0.89$) implying that data collected from the experiment at higher temperatures
233 could reasonably be used to extrapolate from the end of the low-temperature
234 experiment. Higher temperatures led to higher SGR, varying from 5.93 ± 0.35 % TW d⁻¹
235 at 24 °C to 2.60 ± 0.08 % TW d⁻¹ at 11 °C (Table 1). Differences were significant
236 amongst all of the temperatures (Tukey Tests, $p<0.01$) with the exception of both values

237 recorded at 20 °C. Accordingly, median development time (MDT) showed an identical
238 pattern, varying significantly (ANOVA, $F=86.03$, $p<0.01$) from 25 days at 11 °C to 10
239 days at 24 °C, with no differences at 20 °C (Tukey Test, $p=0.97$).

240 Larvae increased in size with time (Fig. 1) despite a different SGR for each temperature
241 (Table 1), to a stabilisation point that coincided with the appearance of cyprids. Once
242 cyprids comprised >50% of the culture, average larval greatest width (LGW) decreased
243 slightly due to the smaller size of cyprids compared to nauplius VI. No significant
244 differences were detected in cyprid carapace width (CW) for cultures reared at different
245 temperatures (low temperatures: ANOVA, $F=0.31$, $p=0.73$; high temperatures;
246 ANOVA, $F=0.25$, $p=0.81$; and overlapping temperature of 20 °C, ANOVA, $F=0.27$;
247 $p=0.79$). CW averaged 209.03 ± 1.92 μm . Larval stage progression index (SPI) gave a
248 clearer view of stage dominance (Fig. 2), particularly when the majority of the larvae
249 were cyprids. At this point, high mortality was observed if no appropriate settlement
250 substrata were provided. Asynchronous development was more noticeable in cultures
251 raised at lower temperatures (e.g. 11–15 °C).

252 Larval survival decreased steadily with time at all temperatures (Fig. 3). At low
253 temperatures, daily mortality rate did not vary significantly according to temperature
254 (ANCOVA, $F=2.01$, $p=0.14$), however overall survival was significantly different
255 (ANOVA, $F=59.10$, $p<0.01$) due to differences in MDT. However, temperatures ≥ 20
256 °C had a significant effect on daily mortality rate (ANCOVA, $F=5.43$, $p<0.01$) in
257 addition to the expected effect on survival (ANOVA, $F=64.55$, $p<0.01$). When the
258 survival results from both temperature experiments were analysed together, significant
259 differences were evident (ANOVA, $F=1.59$, $p<0.03$), while both 20 °C treatments
260 produced similar results (Tukey Test, $p=0.99$). Survival was significantly lower (Tukey
261 Test, $p<0.01$) at 11, 22 and 24 °C (not significantly different; Tukey Test, $p\geq 0.89$)

262 compared to 15 and 20 °C (not significantly different; Tukey Test, $p \geq 0.99$; see Table 1).
263 Survival increased significantly with increasing temperature until optimal conditions
264 were achieved (Table 1), from 10.81 ± 5.26 % at 11 °C to 26.99 ± 4.56 % at 20 °C
265 (average). Temperatures of 22 and 24 °C led to mortalities above 90 %, equivalent to
266 less than 250 surviving larvae per replicate. It is noted that the sampling process could
267 have decreased the estimate of total survival by up to 15 % in the various treatments,
268 depending on the median development time and respective sampling pressure.

269 In a similar way to the difference in survival, there were significant differences in the
270 percentage of high-quality cyprids produced at different temperatures (H-Q cyprids;
271 ANOVA, $F=16.69$, $p < 0.01$). This was significantly higher (Tukey Test, $p < 0.03$) at 15
272 °C and both 20 °C treatments (not significantly different, Tukey Test, $p \geq 0.57$; average
273 of 13.46 ± 0.89 % of initial larvae) in comparison to larvae reared at 11, 22 and 24 °C
274 (not significantly different, Tukey Tests, $p \geq 0.99$; average of 5.07 ± 0.59 % of initial
275 larvae). There were no differences in the percentage of high-quality larvae collected (H-
276 Q larvae; ANOVA, $F=2.84$, $p=0.06$), on average 62.33 ± 0.94 %, or cyprids (ANOVA,
277 $F=1.62$, $p=0.23$), on average 89.22 ± 1.20 %.

278 3.2 Diet

279 There were no significant differences in SGR (ANOVA, $F=1.87$, $p=0.17$) according to
280 diet (Table 2). LGW and SPI showed similar patterns of change over time between
281 treatments (data not shown). Similarly, CW was the same irrespective of larval diet
282 (ANOVA, $F=0.41$, $p=0.65$; Table 2). Average SGR varied between 3.17 and 3.78 %
283 $TW d^{-1}$ (Table 3). The differences observed had only a minor influence on MDT, which
284 varied between 15 and 16 days (Table 3) and were not significantly different between
285 diets (ANOVA, $F=0.23$, $p=0.88$).

286 Due to the lack of differences in MDT, sampling had a similar impact across treatments,
287 where approximately 9 % of larvae were sampled. Survival varied significantly with
288 diet (ANOVA, $F=9.93$, $p<0.01$) (Table 2). The significant differences in total survival
289 were also reflected in similar differences in daily mortality. Larvae grown on diets of *T.*
290 *suecica*, *T. suecica/S. marinoi* and *I. galbana/S. marinoi* had significantly higher
291 survival (Tukey, $p\leq 0.01$); although the mixed diets did not differ significantly from each
292 other in supporting successful development of larvae (Tukey, $p\geq 0.99$). The lowest
293 survival (Tukey, $p\leq 0.01$) was recorded with diets of *I. galbana*, followed by *T. suecica/*
294 *I. galbana* and *S. marinoi*, which were not significantly different (Tukey, $p\geq 0.99$; Table
295 2). Mortalities were noted in the first day of culture on the diet of *I. galbana*, compared
296 to more gradual mortality for the other diets, with survival decreasing steadily with
297 time. Independently, none of the diets tested was toxic to the larvae and all were capable
298 of sustaining development beyond nauplius stage II (the first feeding stage), in spite of
299 different degrees of success.

300 Interestingly, when larvae were assessed for quality (Table 3), there were significant
301 differences between treatments (ANOVA, $F=89.92$, $p<0.01$). Diets of *S. marinoi* and *I.*
302 *galbana/S. marinoi* led to a higher percentage of H-Q larvae (69.67 ± 7.09 and $63.67 \pm$
303 6.51 %, respectively; not significantly different; Tukey, $p=0.74$), followed by *T.*
304 *suecica/S. marinoi* (not different from *I. galbana/S. marinoi*; Tukey, $p=0.17$), and *T.*
305 *suecica* and *T. suecica/I. galbana* (not different from each other; Tukey, $p=0.95$), with
306 *I. galbana* leading to the lowest larval quality (Tukey, $p<0.01$), with no H-Q larvae
307 observed. Similarly, the percentage of larvae that developed to the cyprid stage varied
308 significantly with diet (ANOVA; $F=179.36$; $p<0.01$). This was higher for mixed diets of
309 *T. suecica* and *I. galbana* with *S. marinoi* (90.33 ± 4.00 %; inter-diet comparison not
310 significantly different; Tukey, $p=1.00$), followed by monodiets of *S. marinoi* ($49.67 \pm$

311 1.45 %; Tukey, $p < 0.01$; Table 3) and *T. suecica*/*I. galbana* (32.67 ± 3.48 %; Tukey,
312 $p < 0.01$). The lowest percentage of cyprids was observed in cultures reared on
313 monodiets of *T. suecica* (9.67 ± 2.03 %), with no cyprids observed in cultures reared on
314 *I. galbana* (Table 3). Most importantly, the final number of H-Q cyprids, obtained from
315 the initial larvae, was significantly different (ANOVA, $F=24.39$, $p < 0.01$) for cultures
316 reared with mixed diets containing *S. marinoi*, either provided together with *I. galbana*
317 or *T. suecica*, where respectively 15.15 ± 2.62 % and 11.32 ± 4.66 % of initial nauplii
318 became H-Q cyprids. The remaining diets produced, on average, less than 2 % of
319 cyprids from the initial stock (Table 3).

320 3.3 Photoperiod

321 Photoperiod affected larval SGR significantly (ANOVA, $F=223.47$, $p < 0.01$) and
322 consequently MDT to the cyprid (ANOVA, $F=17.18$, $p < 0.01$). SGR varied on average
323 between 4.03 and 5.27 % TW d^{-1} (Table 4). Larvae grown on a 24:0 L:D cycle had the
324 highest SGR, though not significantly different from a 16:8 L:D (Tukey, $p=0.79$),
325 followed by 8:16 L:D, while the 0:24 L:D treatment had the lowest growth rate (all
326 significantly different; Tukey, $p < 0.01$). The increase in LGW with time is noticeable in
327 Figure 4a and correlates with differences in the MDT to the cyprid (Table 4) and SPI
328 (Fig. 4b). There was, however, no significant difference in CW (ANOVA, $F=0.35$,
329 $p=0.71$). Furthermore, larvae grown in full-day photoperiod developed into cyprids in
330 14 days, significantly faster than other photoperiods (Tukey Test; $p \leq 0.03$). Results from
331 MDT for long and short-day photoperiods were not significantly different from each
332 other (Tukey Test; $p=0.96$) and the longest development time (17 days) was in full-
333 darkness. The early appearance of nauplius VI and cyprids in these cultures (clearly
334 visible after day 11, approximately 4 days in advance of the other photoperiods (Fig. 4
335 a, b) was also consistent with the shorter development time in full-illumination.

336 Nevertheless, though survival varied between treatments, these differences were not
337 significant (ANOVA, $F=2.39$, $p=0.08$), in spite of the markedly different development
338 times (with sampling removing 9–10 % of larva). Cultures grown at 0:24 L:D, 16:8
339 L:D, 24:0 L:D and 8:16 L:D had survival of 21.94 ± 5.45 %, 24.44 ± 4.12 %, $27.00 \pm$
340 3.01 % and 37.33 ± 4.42 %, respectively (Table 4).

341 There were no significant differences between cultures maintained under different
342 photoperiods with respect to the percentage of H-Q larvae (ANOVA, $F=1.57$, $p=0.27$),
343 percentage of cyprids (ANOVA, $F=3.02$, $p=0.09$) and percentage of H-Q cyprids
344 (ANOVA, $F=2.52$, $p=0.13$), which were 60.00 ± 1.90 %, 87.25 ± 1.69 % and $14.82 \pm$
345 0.84 % of the initial number of larvae, respectively.

346 3.4 Salinity

347 No effects of salinity were observed (20, 30 and 40 gL^{-1}) on larval SGR (ANOVA,
348 $F=0.66$, $p=0.52$), survival (ANOVA, $F=0.87$, $p=0.42$), MDT to the cyprid (ANOVA,
349 $F=0.13$, $p=0.98$), or CW (ANOVA, $F=0.22$, $p=0.91$), with approximately 8 % of larvae
350 sampled in total per replicate. The results are summarised in Table 5. SGR, total
351 survival and MDT averaged 4.18 ± 0.05 % d^{-1} , 24.26 ± 3.89 %, and 15 days,
352 respectively. LGW and SPI showed similar variation with time, independent of
353 treatment (data not shown). There were no significant effects of salinity on the
354 percentage of H-Q larvae (ANOVA, $F=1.29$, $p=0.34$), percentage of cyprids (ANOVA,
355 $F=2.81$, $p=0.14$) or percentage of H-Q cyprids (ANOVA, $F=0.25$, $p=0.79$), which were
356 similar to the other experiments investigating photoperiod and temperature (data not
357 shown).

358 4. Discussion

359 4.1 Temperature

360 Temperature had a significant effect on specific growth rate (SGR), as had been
361 reported previously for larvae of *P. polymerus* (Lewis, 1975), *C. mitella* (Zhang *et al.*,
362 2009; Rao *et al.*, 2010) and *P. pollicipes* (Coelho, 1990). The differences in SGR can be
363 explained by the effects of increasing temperature on metabolism. The literature
364 suggests a minimum development time for *P. pollicipes* from hatching to the cyprid of
365 23 to 28 days at 20 °C (Molares *et al.*, 1994b), 14 days at 17.5 °C (to nauplii VI;
366 Candeias, 2005) and 11 to 24 days (average 15 days) at 15 to 24 °C (Kugele & Yule,
367 1996). In the present study, medium development time (MDT) ranged from 10 to 25
368 days between 24 °C and 11 °C, and was 15 to 16 days at 20 °C. These results are in line
369 with the previous reports, except for the study by Molares *et al.* (1994b), where
370 development time was longer. This discrepancy was most likely due to considerable
371 differences in feeding quantity and quality between the two studies. The differences in
372 SGR with temperature, reflected in lower development times at higher temperatures, are
373 in accordance with Coelho (1990); to our knowledge the only study to date on the effect
374 of temperature on *P. pollicipes* larval growth. Coelho (1990) reported that the duration
375 of development from nauplius stage I to nauplius VI decreased from 20 days at 15 °C to
376 9 days at 22 °C, but no details were provided of specific growth rate or survival. Rather,
377 the time of appearance of each stage was reported, which provides limited information
378 about culture performance. Similarly, for *C. mitella* with its longer larval phase (Qiu *et*
379 *al.*, 1994a), higher temperatures have been reported to decrease development time from
380 11 days (at 24 °C) to 7 days (at 30 to 31 °C) (Zhang *et al.*, 2009; Rao *et al.*, 2010).

381 There was a direct relation between SGR and survival at the lower temperature range, as
382 the higher the SGR and SPI, the lower the overall mortality. However, at higher
383 temperatures (>20 °C), daily mortality rate increased with temperature, creating an
384 opposing trend. This is in accordance with Lewis (1975) for *P. polymerus*, who

385 suggested that higher temperatures can increase the growth rate, while fitness and
386 survival. Likewise, Coelho (1990) reported mass mortality at 22 °C. The question
387 therefore remains, what would be the highest temperature at which the decrease in
388 development time does not correlate with an increase in mortality? Overall survival
389 varied between 19 to 31 % at an optimal temperature range of 15 to 20 °C, to values
390 below 11 % at 11 °C and above 22 °C. Temperatures between 14 to 20 °C are within the
391 range normally experienced by this species in its natural habitat, from May to September
392 (Sines, Portugal; Instituto Hidrográfico 2015), during the breeding season (Cruz 2000).
393 The fact that *P. pollicipes* survival dropped when reared above 20 °C and virtually no
394 cyprids developed at 24 °C, raises concerns over using temperatures above 20 °C. Lower
395 survival at higher temperatures could also be ascribed to a putative decrease in water
396 quality at these temperatures or eventual proliferation of contaminating species, as
397 reported by Candeias (2005). From our experience, most of the culture problems
398 resulted directly from the long development time (a minimum of 15 days at 20 °C). This
399 is much longer than commonly cultured barnacles, such as *B. amphitrite* and *B.*
400 *improvisus*, whose cyprids can be obtained in 4 to 5 days (28 °C; Franco, pers. obs.). In
401 fact, several authors (Molares, 1994; Candeias, 2005; Coelho, 1990) have reported very
402 high mortality (≥ 85 %) during the first two weeks of rearing, which highlights the
403 importance of decreasing the rearing period for this species and improving culture
404 conditions. Furthermore, the effect of sampling in the present study also impacted the
405 measure of survival leading to a slight underestimation. Although each replicate would
406 ideally have been stocked initially with sufficient larvae for the impact of sampling to
407 be negligible, available methods for larval collection were limiting. A very large
408 number of wild-collected adults would also have been required, as these in turn often

409 showed high variability in maturity and contained egg lamellae at different stages of
410 development.

411 While a clear indication of best rearing temperature was obtained, survival was poor
412 compared to *C. mitella*, for which total survival to the cyprid ranged from 90 to 99 %
413 and metamorphosis from 73 to 81 % at the optimal temperature of 27 °C (Rao *et al.*
414 2010). For *P. pollicipes*, close monitoring of cultures is also required so that they can be
415 filtered when there are >50 % cyprids, as high mortality can occur and cyprid quality
416 can decrease abruptly at this point. Results for other species (e.g. *B. amphitrite*; Costlow
417 & Bookhout, 1959) also suggest that the transition from the last nauplius stage to the
418 cyprid is a particularly vulnerable point in the life cycle, with increased mortality
419 occurring prior to the cyprid stage.

420 4.2 Diet

421 Feeding and nutritional requirements are of paramount importance to the successful
422 larval culture of *Pollicipes* spp. (Moyses, 1963; Lewis, 1975). The only studies to
423 investigate larval diet were conducted by Lewis (1975) for *P. polymerus*, and by Coelho
424 (1990) and Candeias (2005) for *P. pollicipes*. For *P. pollicipes*, consumption rate varied
425 according to algal species provided as food (Candeias, 2005). From the diets tested in
426 the present study, no significant differences were detected for SGR and MDT, although
427 monodiets of *T. suecica* gave the highest growth rates. Diet did not significantly
428 influence growth and development time, but it did significantly affect survival, which
429 ranged from 13 to 40 %. Unlike the results from previous studies (Coelho, 1990), all the
430 diets tested allowed progression through the naupliar stages, though not necessarily to
431 the cyprid and the production of high-quality cyprid larvae (H-Q cyprids). Inadequacy
432 of diet may be due to poor nutritional profile and algal bioavailability, with inadequate
433 feeding causing mechanical interference with swimming (e.g. live preys become trapped

434 in the larval appendages), the accumulation of lethal metabolites, deficient development
435 and precocious death (Franco, pers. obs.; Moyse, 1963; Lewis, 1975).

436 Highest survival was attained with diets of *T. suecica* alone, and mixed diets of *T.*
437 *suecica* or *I. galbana*, with *S. marinoi*. Furthermore, the larvae fed with the *S. marinoi*
438 monodiet and mixed diets containing this species had the highest percentage of high-
439 quality larvae (H-Q larvae) and survival to the cyprid. Thus, while *S. marinoi* alone
440 proved sub-optimal for larval development, when it was used in conjunction with *T.*
441 *suecica* or *I. galbana* it gave good results with respect to larval growth and survival to
442 the cyprid. Candeias (2005) concluded that all *P. pollicipes* larval stages show a
443 preference for ingesting *I. galbana* and *S. costatum* and, to a lesser extent, *T. suecica*.
444 Additionally, mixed diets and flagellate-based diets (*Rhinomonas reticulata*) produced
445 higher survival than monodiets of diatoms, namely *S. marinoi* (Candeias, 2005), as also
446 observed in the present study. Despite the general preference of temperate species for
447 flagellates (Moyse, 1963; Stone, 1988), Candeias (2005) suggested that *P. pollicipes*
448 might have developed a propensity for feeding on diatoms due to seasonal upwelling.

449 In spite of the poor survival results obtained in this experiment with mixed diets of *T.*
450 *suecica*/*I. galbana*, results from the other experiments undertaken in similar conditions
451 where the same diet was used (e.g. experiments 1 and 3, for treatments cultured in
452 similar conditions) recorded an average higher survival (20 to 30 % survival) for this
453 diet. Independently, the poor survival results here observed with *I. galbana* are in
454 accordance with results from previous experiments.

455 Coelho (1990) also concluded that from monodiets of *S. costatum*, *T. suecica*,
456 *Thalassiosira pseudonana*, *I. galbana*, *Chaetoceros gracilis* and *Chorella* sp., only *T.*
457 *suecica* and *I. galbana* supported larval development to the cyprid, with remaining

458 monodiets not sustaining development beyond nauplius stages II and III. This
459 contradicts the results reported here, where monodiets of *S. marinoi* also sustained
460 development to the cyprid stage. However, none of the diets used in this study resulted
461 in more than 15% of nauplii surviving to H-Q cyprids so there is considerable scope for
462 improvement.

463 Optimising the diet of *P. pollicipes* larvae is essential to close the life cycle of this
464 species in culture as settlement may otherwise be compromised. Settlement of *B.*
465 *amphitrite* cyprids was lower, for example, when reared on *Dunaliella tertiolecta*
466 compared to *S. costatum*. (Clare *et al.* 1994). From the current results, mixed diets
467 containing *S. marinoi* produced maximal survival, H-Q larvae and percentage of
468 cyprids. Therefore, future studies could investigate effects of diet on cyprid settlement,
469 providing that suitable settlement surfaces can be identified; *P. pollicipes* being averse
470 to settling on artificial surfaces in captivity (e.g. Coelho, 1991; Kugele & Yule, 1996).

471 4.3 Photoperiod

472 Although there have been no studies into the effects of light exposure on *P. pollicipes*
473 larval growth and survival, there have been studies of how light affects behaviour
474 (Molares *et al.*, 2002). *P. pollicipes* larvae are phototactic and Molares (1994)
475 established that most of the time this will manifest as positive phototaxis. This
476 characteristic has long been used for selection of the healthiest larvae, by attraction to
477 light. The prevailing choice in previous studies has been to use natural photoperiod (e.g.
478 Coelho, 1990) or full day photoperiod (Molares 1994; Molares *et al.*, 1994b), due to the
479 lack of supporting evidence for any other photoperiod being preferable. In the present
480 study, larval growth was affected by photoperiod but there was no significant effect on
481 survival. Larvae grown in 24:0 L:D, short and long day photoperiod had the highest
482 SGR. Longer-day photoperiods can directly promote algal proliferation, allowing for

483 greater food availability for an extended period of time, which may contribute to faster
484 growth in the full-light photoperiod. In the Crustacea, there is high variability in
485 response to photoperiod during larval rearing (e.g. *Strombus pugilis*, Andrés *et al.*,
486 2010; *Portunus pelagicus*, Bermudes & Ritar, 2008; *Jasus edwardsii*, Manzano *et al.*,
487 1998) and therefore species-specific protocols are required. The present results support
488 the use of longer-day photoperiods, which is in accordance to what has been used to
489 date by previous authors. Furthermore, it is interesting to note that during the peak of *P.*
490 *pollicipes*' breeding season (June to August) temperature normally approximates 20 °C
491 and photoperiod ranges between 14 – 15 hours of light, agreeing with the present results
492 for best larval performance.

493 4.4 Salinity

494 The effect of salinity on the growth and survival of marine invertebrate larvae has
495 received considerable attention. Unnatural regimes have proven beneficial in species of
496 bivalves, crustaceans and fish, and several authors working with larvae (e.g. *Penaeus*
497 *semisulcatus*, Innes & Haley, 1977; *Mytilus edulis*, Fonds, 1979; *Solea solea*. Kumlu *et*
498 *al.*, 2000) have shown that higher salinities, within a tolerance range, tend to enhance
499 growth. Lower salinities, on the contrary, often improve survival. In the case of *P.*
500 *pollicipes* larval culture, salinities reported in the literature have usually been within the
501 natural range of salinities 33 to 34 gL⁻¹ (Coelho, 1990; Molaes, 1994; Molaes *et al.*,
502 1994a). The effect of salinity on a species will depend largely on the ability to
503 osmoregulate at early stages of development (e.g. Charmantier, 1998) and whether or
504 not there are effects on the biochemical composition of the larvae such as lipid reserves
505 (e.g. Torres *et al.*, 2002). In the present study, there was no effect of salinity on larval
506 survival, SGR, MDT and CW, in the range 20 to 40 gL⁻¹, indicating that *P. pollicipes* is
507 highly tolerant within this range, in spite of the salinities tested spanning beyond the

508 ones found in the natural habitat. In the wild, *P. pollicipes* adults are normally found at
509 salinities of 33 to 37, although salinity in the intertidal environment can vary
510 significantly due to evaporation in the hours of air exposure and rainfall. In spite of the
511 limitations of this work, where the interactions of factors (e.g. temperature vs. salinity)
512 were not studied due to insufficient larval numbers, results suggest that *P. pollicipes* can
513 survive throughout this range.

514 **5. Conclusions**

515 Based on the present results, improved growth and survival can be accomplished using
516 rearing temperatures of 15 to 20 °C, daily feeding with *T. suecica/S. marinoi* or *I.*
517 *galbana/S. marinoi* and a photoperiod of 24:0 L:D. The use of higher temperatures (22
518 and 24 °C) significantly increased mortality and reduced the number of cyprids, while
519 lower temperatures (11 to 20 °C) extended the growth period, which led to higher
520 mortality. In practical terms, the decision as to which temperature to use within this
521 range (15 to 20 °C) should rely upon the relative benefits of having a longer or shorter
522 growth period in any given situation. In spite of no differences in specific growth rate
523 with regard to diet, *T. suecica/S. marinoi* or *I. galbana/S. marinoi* assured the best
524 results in terms of the percentage of high-quality cyprids produced, therefore
525 maximising rearing efficiency. Photoperiod, on the other hand, affected growth with
526 better results in 24:0 and 16:8 L:D photoperiods, but with median development time
527 reduced by full-day photoperiods. A 24-h period of darkness should be avoided. No
528 effects were observed with regard to salinity, suggesting that *P. pollicipes* larvae
529 tolerate a wide range of salinities. In order to improve water quality and reduce
530 maintenance costs, while decreasing handling of larvae, further research could focus on
531 the use of recirculating systems for larviculture. As there are currently no viable
532 protocols for ensuring settlement of *P. pollicipes* larvae in the laboratory (see Kugele &

533 Yule, 1996), it would also be prudent to build upon the present results and further
534 investigate the implications of larval culture conditions towards competence to settle
535 and ability to complete metamorphosis.

536

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546

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672

673 **Figure legends**

674 Fig. 1 Average larval greatest width (μm) according to culture time (days), for larvae
675 grown at: a) low temperatures (11, 15, 20 °C) and b) high temperatures (20, 22 and 24
676 °C). Values are presented as mean \pm SE.

677

678 Fig. 2 Larval stage progression (#) according to culture time (days), for larvae grown at:
679 a) low temperatures (11, 15, 20 °C) and b) high temperatures (20, 22 and 24 °C). Stages
680 were assigned as follows: 1) nauplii I, 2) nauplii II, 3) nauplii III, 4) nauplii IV, 5)
681 nauplii V, 6) nauplii VI, and 7) cyprids. Values are presented as mean \pm SE.

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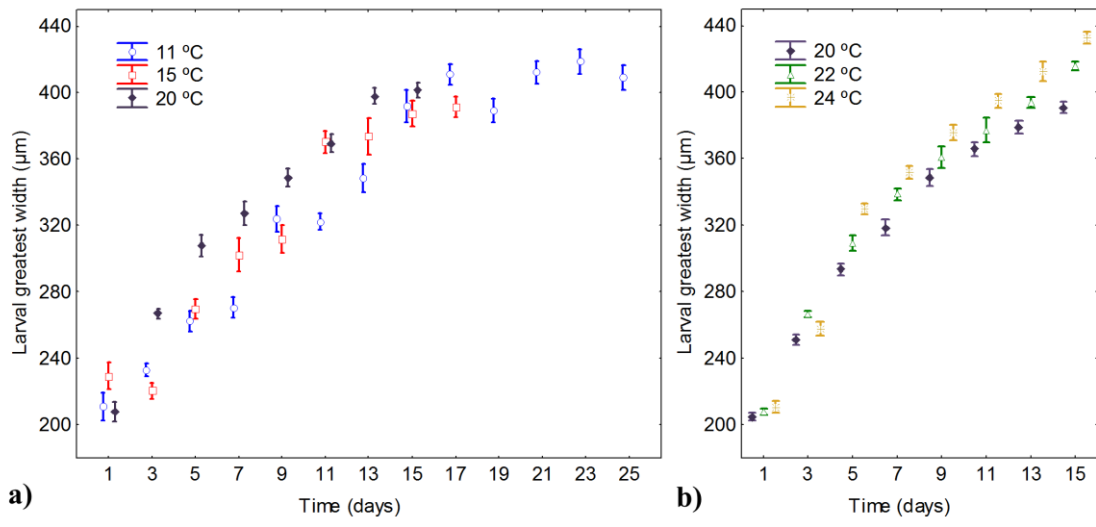
683 Fig. 3 Survival (%) according to culture time (days), for larvae grown at: a) low
684 temperatures (11, 15, 20 °C) and b) high temperatures (20, 22 and 24 °C). Values are
685 presented as mean \pm SE.

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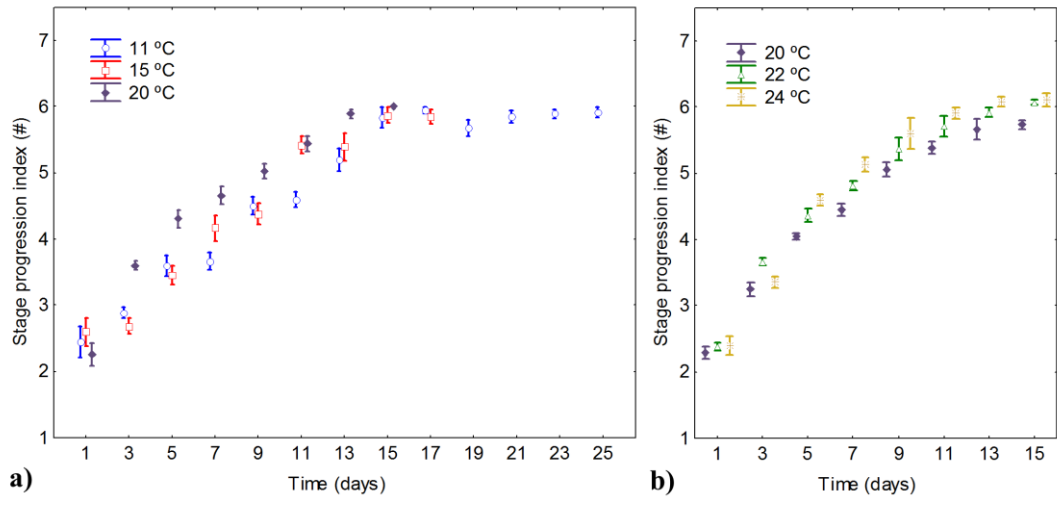
687 Fig. 4 (a) Larval greatest width (μm), and (b) stage progression index (#) according to
688 culture time (days), for larvae grown at four different photoperiods, namely full dark
689 photoperiod (0:24 L:D), 16 h of light and 8 h of darkness (16:8 L:D), 8 h of light and 16
690 h of darkness (8:16 L:D), and full light photoperiod (24:0 L:D). Values are presented as
691 mean \pm SE.

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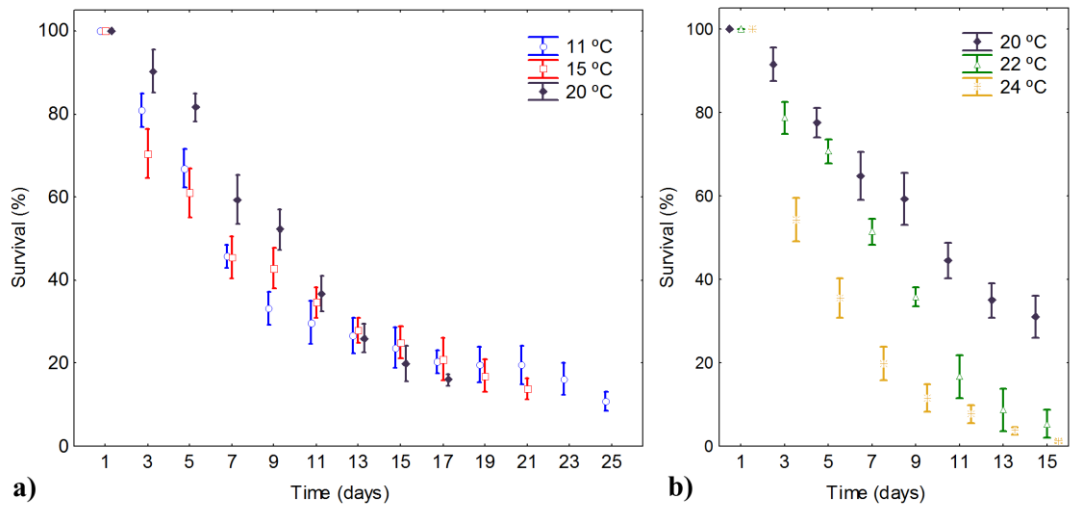
693 **Figures**



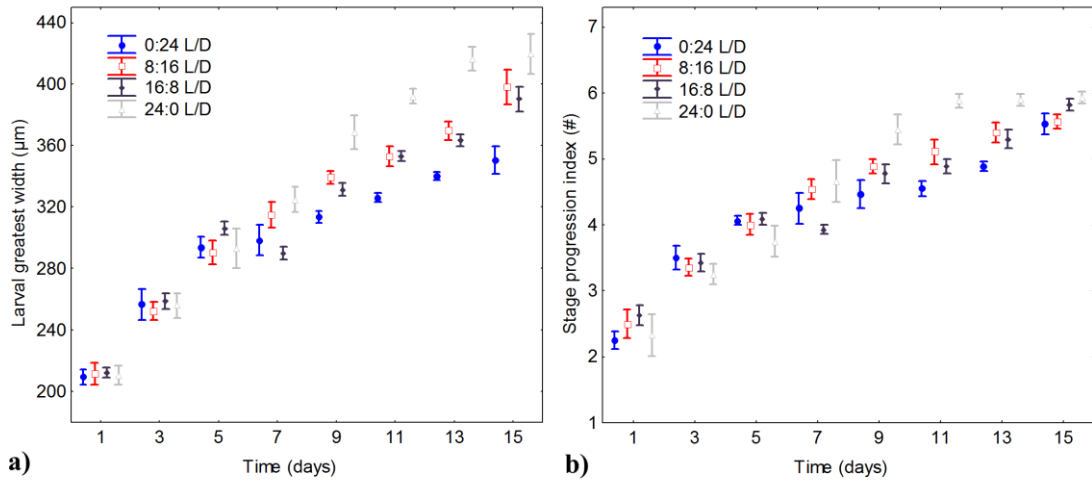
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707 **Tables**

708 Table 1. Specific growth rate (SGR, % d⁻¹), total survival to cyprid (tS, %), median development time to
 709 cyprids (MDT, days) and cyprid width (CW, μm), for larvae grown at low temperatures (11, 15, 20 °C)
 710 and high temperatures (20, 22 and 24 °C). Values are presented as mean ± SE. Different superscripts
 711 indicate significant differences, for each parameter, following ANOVA and Tukey's HSD tests.

Experiment	T (°C)	SGR (% d ⁻¹)	tS (%)	MDT (d)	CW (μm)
Low temp.	11	2.60 ± 0.08 ^a	10.81 ± 5.26 ^a	24.83 ± 0.29 ^a	200.00 ± 7.96 ^a
	15	2.97 ± 0.10 ^b	19.08 ± 2.83 ^b	17.67 ± 0.58 ^b	209.65 ± 3.52 ^a
	20	4.57 ± 0.15 ^c	22.87 ± 3.86 ^b	15.23 ± 0.68 ^c	211.78 ± 2.34 ^a
High temp.	20	4.50 ± 0.14 ^c	31.11 ± 5.26 ^b	16.06 ± 0.39 ^c	205.12 ± 4.52 ^a
	22	5.31 ± 0.47 ^d	8.69 ± 5.38 ^a	13.45 ± 0.51 ^d	208.24 ± 3.83 ^a
	24	5.93 ± 0.35 ^e	7.67 ± 2.29 ^a	9.81 ± 0.25 ^e	203.03 ± 2.54 ^a

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715 Table 2. Specific growth rate (SGR, % d⁻¹), total survival to cyprid (tS, %), median development time to
 716 cyprids (MDT, days) and cyprid width (CW, μm), for larvae cultured using various diets, of *Tetraselmis*
 717 *suecica*, *Isochrysis galbana* and *Skeletonema marinoi*, presented as both mono- and mixed-diets. In
 718 treatments where cyprids were not observed, median development time was classified as *n.a.* - not-
 719 applicable. Values are presented as mean ± SE. Different superscripts indicate significant differences, for
 720 each parameter, following ANOVA and Tukey's HSD tests.

Diet	SGR (% d ⁻¹)	tS (%)	MDT (d)	CW (μm)
<i>T. suecica</i>	3.78 ± 0.28 ^a	38.30 ± 6.65 ^a	14.99 ± 2.54 ^a	212.35 ± 3.62 ^a
<i>I. galbana</i>	3.17 ± 0.38 ^a	13.03 ± 3.03 ^b	n.a.	n.a.
<i>S. marinoi</i>	3.40 ± 0.30 ^a	16.89 ± 2.26 ^b	15.30 ± 2.88 ^a	209.45 ± 2.99 ^a
<i>T. suecica</i> / <i>I. galbana</i>	3.39 ± 0.28 ^a	14.60 ± 3.93 ^b	15.32 ± 3.55 ^a	210.98 ± 3.17 ^a
<i>I. galbana</i> / <i>S. marinoi</i>	3.26 ± 0.24 ^a	38.72 ± 8.60 ^a	16.05 ± 3.18 ^a	207.15 ± 4.03 ^a
<i>T. suecica</i> / <i>S. marinoi</i>	3.43 ± 0.17 ^a	40.82 ± 2.37 ^a	15.27 ± 2.54 ^a	202.86 ± 3.88 ^a

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724 Table 3. Percentage of high-quality larvae (H-Q larvae, %), cyprid larvae (Cyprids, %) and high-quality
 725 cyprids (H-Q cyprids, %) obtained after rearing and larvae collection, for larvae cultured using various
 726 diets, of *Tetraselmis suecica*, *Isochrysis galbana* and *Skeletonema marinoi*, presented as both mono- and
 727 mixed-diets. Values are presented as mean \pm SE. Different superscripts indicate significant differences,
 728 for each parameter, following ANOVA and Tukey's HSD tests.

Diet	H-Q larvae (%)	Cyprids (%)	H-Q cyprids (% n)
<i>T. suecica</i>	14.33 \pm 4.04 ^a	9.67 \pm 2.03 ^a	0.21 \pm 0.18 ^a
<i>I. galbana</i>	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a
<i>S. marinoi</i>	69.67 \pm 7.09 ^c	49.67 \pm 1.45 ^b	1.27 \pm 2.19 ^a
<i>T. suecica</i> / <i>I. galbana</i>	18.00 \pm 5.57 ^a	32.67 \pm 3.48 ^b	0.20 \pm 0.35 ^a
<i>I. galbana</i> / <i>S. marinoi</i>	63.67 \pm 6.51 ^{cd}	90.33 \pm 3.76 ^c	15.15 \pm 2.62 ^b
<i>T. suecica</i> / <i>S. marinoi</i>	52.33 \pm 5.51 ^d	90.33 \pm 4.26 ^c	11.32 \pm 4.66 ^b

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732 Table 4. Specific growth rate (SGR, % d⁻¹), total survival to cyprid (tS, %), median development time to
 733 cyprids (MDT, days) and cyprid width (CW, μm), for larvae cultured at various photoperiods. Values are
 734 presented as mean ± SE. Different superscripts indicate significant differences, for each parameter,
 735 following ANOVA and Tukey's HSD tests.

Photoperiod	SGR (% d ⁻¹)	tS (%)	MDT (d)	CW (μm)
0:24 L:D	4.03 ± 0.05 ^a	21.94 ± 5.45 ^a	16.67 ± 0.33 ^a	206.76 ± 2.76 ^a
8:16 L:D	4.87 ± 0.07 ^b	37.33 ± 4.42 ^a	15.33 ± 0.31 ^b	208.87 ± 3.55 ^a
16:8 L:D	5.22 ± 0.08 ^c	24.44 ± 4.12 ^a	15.13 ± 0.11 ^b	204.45 ± 3.71 ^a
24:0 L:D	5.27 ± 0.07 ^c	27.00 ± 3.01 ^a	13.67 ± 0.29 ^c	211.27 ± 3.82 ^a

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739 Table 5. Specific growth rate (SGR, % d⁻¹), total survival to cyprid (tS, %), median development time to
 740 cyprids (MDT, days) and cyprid width (CW, μm), for larvae cultured at various salinities (20, 30 and 40
 741 psu). Values are presented as mean ± SE. Different superscripts indicate significant differences, for each
 742 parameter, following ANOVA and Tukey's HSD tests.

Salinity	SGR (% d ⁻¹)	tS (%)	MDT (d)	CW (μm)
20 psu	4.20 ± 0.07 ^a	28.61 ± 4.51 ^a	14.35 ± 1.99 ^a	210.33 ± 2.59 ^a
30 psu	4.18 ± 0.06 ^a	23.06 ± 3.81 ^a	14.45 ± 2.08 ^a	212.18 ± 3.62 ^a
40 psu	4.14 ± 0.07 ^a	21.11 ± 4.15 ^a	14.78 ± 1.87 ^a	205.88 ± 4.11 ^a

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