# Influence of temperature and light regime on the larval development of the common spider crab *Maja brachydactyla Balss*, 1922 (Brachyura: Majidae)

Diego Castejón[1, 2]*, Guiomar Rotllant[2], Luis Giménez[3], Gabriela Torres[3,4], and Guillermo Guerao[5]

[1] Cultius Aquàtics, IRTA, Ctra. de Poble Nou, Km 5.5, Sant Carles de la Ràpita 43540, Tarragona, Spain

[2] Departament de Recursos Marins Renovables, Institut De Ciències Del Mar, CSIC, Passeig Marítim de la Barceloneta 37-49, Barcelona 08003, Spain

[3] School of Ocean Sciences, Bangor University, Menai Bridge, Anglesey, LL59 5AB, United Kingdom.

[4] Present address: Alfred Wegener Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Biologische Anstalt Helgoland,27498 Helgoland, Germany

[5]Independent researcher. Barcelona (Spain)

**Short title**: Temperature & light influence on brachyuran larvae

***Corresponding author**: Diego Castejón. E-mail: diego.castejon.dcb@gmail.com; phone: +34 681 154 398; postal address: Departament de Recursos Marins Renovables, Institut De Ciències Del Mar, CSIC, Passeig Marítim de la Barceloneta 37-49, Barcelona 08003, Spain

**Abstract.**

Temperature and light are important factors affecting production in the aquaculture industry, as they can drive behavior and physiological responses of free-swimming larval stages. However, the influence of light on crustacean farming has received little consideration. The common spider crab *Maja brachydactyla* Balss, 1922 has a great potential for aquaculture because of the easy maintenance, high fecundity and short larval development. In order to optimize larval culture techniques, we quantified the influence of temperature and light on larval survival, development and elemental (carbon and nitrogen) body composition. Constant darkness resulted in longer developmental time as compared with daily light photoperiod (6 to 16 light hours). Larvae reared under constant darkness showed also reduced dry mass, carbon and nitrogen content, and C:N ratio. We also found carry-over effects of light conditions: constant darkness experienced during the zoeal stage led to increased developmental time in the megalopa stage. Temperature and light showed additive effects. We optimized the larval culture of *M. brachydactyla* requiring around 14 days from hatch to first juvenile by keeping cultures under 21 ± 1 °C and light sources simulating the daily light cycle.

**Keywords**: Decapoda; larviculture; temperature; photoperiod; light intensity; elemental body composition

## 1. Introduction

Temperature is the most crucial factor influencing production in the aquaculture industry and crustacean farming since it determines survival, metabolic activity, growth rate and time of development (Anger, 2001; Wickins & O'C Lee, 2002; Castejón, Rotllant, Giménez, Torres & Guerao, 2015). Light is another important factor affecting production in the aquaculture industry, due to its impact on biological cycles, feeding, growth, behavior or reproductive strategies (Boeuf & Le Bail, 1999; Villamizar, Blanco-Vives, Migaud, Davie, Carboni & Sánchez-Vázquez, 2011). However, the influence of light on crustacean farming receives little consideration (Wickins & O'C Lee, 2002). Light can drive behavior and physiological responses of free-swimming larval stages of crustacean species (Anger, 2001; Epifanio & Cohen, 2016). The photoperiod and intensity of light influence the feeding behavior (Viherluoto & Viitasalo, 2001; Minagawa, 1994; Rabbani & Zeng, 2005), growth rate (Minagawa, 1994; Hoang, Barchiesis, Lee, Keenan & Marsden, 2003; Ulikowski & Krzywosz, 2004), metabolic activity (Wang, Dong, Dong, Huang, Zhu & Mu, 2004), survival and duration of larval development (Minagawa, 1994; Rabbani & Zeng, 2005; Andrés, Rotllant & Zeng, 2010). Light intensity can also affect the success of settlement and metamorphosis (Thorson, 1964).The effects of light can be modulated by various environmental factors (Epifanio & Cohen, 2016); for instance, the light-response of larvae of many marine species, including some Decapoda, vary in relation to the temperature (Thorson, 1964; Anger, 2001).

Here, we report on results of a series of experiments, manipulating temperature and light conditions, aimed to optimize larval production of the common spider crab *Maja brachydactyla* Balss, 1922. *Maja brachydactyla* is an important marine resource in France, Ireland, Channel Islands and Spain (FAO, 2014). This species reproduces very

easily in captivity and a single female can carry multiple and consecutive broods (González-Gurriarán, Fernández, Freire & Muiño, 1998). The female fecundity is also very high (range 100,000-500,000 eggs per female depending on environmental and animal conditions: Verísimo, Bernárdez, González-Gurriarán, Freire, Muino & Fernández, 2011; Rotllant, Simeó, Macià & Estévez, 2015). The life cycle has been closed in captivity requiring 20-30 days to complete the larval development at 14-17 °C (Pazos, Fernández, Linares, Sánchez, Otero, Iglesias & Domingues, 2018). The larval development of *M. brachydactyla* is abbreviated, comprising two zoeal stages (zoea I and zoea II) and a megalopal stage (Clark, 1986; Guerao, Pastor, Martin, Andrés, Estévez, Grau, Duran & Rotllant, 2008). Overall, the profitable economic value, easy maintenance and reproduction, high female fecundity, short larval development and feasible larval culture give a great potential for *Maja brachydactyla* to become an aquaculture species. Here, we provide the combination of temperature and light conditions that maximise larval survival and body mass while minimising development time.

## Material and methods

Adult crabs were captured in the Galician coast (North Spain) in December 2012, then were transported to the Institut de Recerca i Tecnologia Agroalimentàries facilities (Sant Carles de la Ràpita, Tarragona, Spain). Crabs were maintained in a sex ratio of six females per male and kept in 2,000 L cylindrical tanks connected to a recirculation unit (renewal rate = 3.5 $m^3$ $h^{-1}$). Animals were kept under constant conditions of temperature (18 ± 1 °C), salinity (35 ± 1), photoperiod (12 h light: 12 h dark) and light intensity (25 lx ≈ 0.75 µmoles $m^{-2}$ $s^{-1}$, fluorescent tubes), and fed with mussels and frozen crabs.

Larvae were reared in glass beakers (volume = 600 mL) filled with 500 mL of filtered seawater (salinity at 35 ± 1; portable handheld salinity refractometer, precision: 1, range: 0 – 100; Shenzhen Handsome Technology Co. Ltd., Guandong, China). Each beaker was used as a single replicate. The beakers were placed in 200 L incubation chambers (100 x 40 x 50 cm) where temperature and light conditions were manipulated. Chamber base and walls were covered with black plastic polyethylene sheets to prevent the influence of external light sources; for treatments of constant darkness the top of the corresponding chambers were covered with black sheets. For treatments with light exposure, illumination was provided from above (0.4 m over the glass beakers). Fluorescent tubes (model 120 220 W, Beta Acuarios, Spain) and white LED lights (model LD65B 6.24 W and model LD100B 14.40 W, ICA, Spain) were used depending on the experiment (see "Experimental design" section for details). The emission spectrum of the light sources was provided from the manufacturer (Supplementary Figure. 1).

Each experiment used larvae from the same hatch and larvae from the same hatch were used for a single experiment. Therefore, the different experiments used larvae from different hatches. Only actively swimming larvae were used for experiments. Each experiment consisted of two phases: 1) The first phase started with freshly hatched zoea larvae (maximum time since hatching = 15 h) reared under the treatment conditions (starting density = 60 zoeae $L^{-1}$: cf. Andrés, Estévez, Anger and Rotllant 2008) until larvae either molted to megalopa or died. 2) The second phase was based on newly molted megalopae (starting density = 20 megalopae $L^{-1}$; maximum time since molting = 15 h) which were then reared until they metamorphosed to first juvenile or died. Megalopa were transferred to the same conditions used during the first phase, except for

an experiment where we studied the effect of photo regime on development from megalopa to juvenile (experiment 4).

Food consisted in fresh nauplii and metanauplii of *Artemia* sp. (INVE Aquaculture Nutrition, Salt Lake UT, USA) without enrichment. Water and food (*ad libitum*) were changed daily and dead larvae were discarded. The criteria used to confirm death was the absence of appendage and heart movement employing a stereomicroscope.

**Experimental designs**

Experiments 1-3 were designed to explore larval responses to temperature, photoperiod and light intensity over a wide range of values; they focus on each factor separately. Experiments 4 and 5 were carried out in order to better understand the influence of temperature and light within a constrained range of parameter values, based on results of experiments 1-3.

1. *Temperature experiment.* Larvae were reared in separate groups under six treatments: 15, 18, 21, 24, 27, and 30 ± 1 °C. Four replicates were established to measure survival and duration of larval development. Four additional replicates were established exclusively for sampling for DM and CHN analyses. Temperature was manipulated using calibrated submersible heaters controlled by an internal thermostat (Eheim Jäger, Finsterrot, Germany) and a water bath for heat distribution. Temperature was measured daily with a portable meter (precision: 0.1 °C; WTW ProfiLine Oxi 3210, Weilheim, Germany). Fluorescent tubes provided illumination. Photoperiod (12 h light: 12 h dark) and light intensity (300 lx ≈ 9 µmoles $m^{-2}$ $s^{-1}$) were kept constant.

2. *Photoperiod experiment.* Five daily light: dark cycle regimes were used: 0, 8, 12, 16 and 24 light hours. Four replicates were established to measure survival and duration of larval development. Four additional replicates were established exclusively for

sampling for DM and CHN analyses. Fluorescent tubes were used to establish the most suitable photoperiod following a previous photoperiod study (Andrés, Rotllant & Zeng 2010). Photoperiod was controlled with 24 hours programmable timers. In this experiment, and in subsequent experiments, during daily maintenance the larvae reared under constant darkness were exposed to the room illumination for 15 – 30 minutes every day, but the term "constant darkness" is used for simplicity. Light intensity (300 lx ≈ 9 µmoles $m^{-2}$ $s^{-1}$) and temperature (21 ± 1 °C) were kept constant.

3. *Light intensity experiment.* Four treatments of light intensity were used: 0, 300 lx (≈ 4.2 µmoles $m^{-2}$ $s^{-1}$), 1000 lx (≈ 14 µmoles $m^{-2}$ $s^{-1}$) and 3000 lx (≈ 42 µmoles $m^{-2}$ $s^{-1}$). Four replicates were established to measure survival and duration of larval development. Four additional replicates were established exclusively for sampling for DM and CHN analyses. LED lights were employed due to their versatility (the number of LED bulbs can be adjusted to modify light intensity). Light intensity was quantified with a light meter (precision: 1 lx; Lx-101, Lutron Electronic Enterprise, Taipei, Taiwan). The conversion to µmoles $m^{-2}$ $s^{-1}$ used conversion tables (http://www.egc.com). The 0 lx treatment corresponded to constant darkness photoperiod while the light treatments were based on 12 h light:12 h dark photoperiod. Temperature (21.0 ± 0.5 °C) was kept constant.

4. *Effect of photo regime on development from megalopa to juvenile.* Only two light regimes were used: constant darkness (D: 0 lx; 0 h light: 24 h dark) and light: dark cycle (L: 1000 lx ≈ 14 µmoles $m^{-2}$ $s^{-1}$; 12 h light: 12 h dark; LED lights). The choice of these regimes was based on previous experiments, where the most significant factor was presence (L)/absence (D) of light rather than variations in photoperiod or intensity (see Results). These conditions were combined in four treatments: DD, DL, LD and LL (first letter: light regime used during the zoeal phase; second letter: light regime used during

164 the megalopal stage). Six replicates per treatment were established to measure survival
165 and duration of larval development. Temperature (19.0 ± 0.5 °C) was kept constant.

166 5. *Combined effects of light regime and temperature on survival and duration of the*
167 *larval development.* Larvae were kept under combinations of two light regimes:
168 constant darkness (D: 0 lx; 0 h light: 24 h dark) and light: dark cycle (L: 1000 lx ≈ 14
169 µmoles $m^{-2}$ $s^{-1}$; 12 h light: 12 h dark; LED lights); combined with two temperatures (18
170 and 21 ± 1 °C) in a factorial design. Four replicates per treatment were used during the
171 zoeal phase. Due to technical problems, in the treatments 'L–18 °C' and 'D–21 °C' only
172 two and three replicates were used respectively during the megalopal stage. The
173 replicates were established to measure survival and duration of larval development.

174 **Dry mass and elemental body composition analysis**

175 The dry mass (DM) and elemental body composition (Carbon: Hydrogen: Nitrogen,
176 CHN) were analyzed in the *Temperature*, *Photoperiod*, and *Light intensity* experiments.
177 In each experiment we sampled randomly 25 newly hatched larvae before the start of
178 the experiment. Four culture beakers were used to obtain five replicate samples for
179 determination of dry mass, carbon and nitrogen of inter-molt zoea I and megalopa. For
180 the zoeae, each replicate required 5 specimens obtained by taking randomly a single
181 larvae per beaker, the fifth zoea was obtained from one of the four beakers randomly
182 selected. For the megalopa, each replicate required 3 specimens obtained by taking
183 randomly a single larvae from three randomly selected beakers. In each replicate, the
184 larvae were pooled into a single group that was subsequently processed for elemental
185 analysis.

186 Hence, each replicate measurement of dry mass, carbon and nitrogen was carried out
187 with larvae from all four beakers. The sampled larvae were rinsed carefully in distilled

water for a few seconds and the excess of water was absorbed with filter paper. Then, larvae were introduced inside tin capsules and stored at -20 °C following Anger and Harms (1990). Samples were freeze-dried (vacuum drier: Edwards Super Modulyo) and the DM was determined (microbalance: Mettler Toledo MX5, precision: 1µg, capacity: 5.1g). The content of CHN was measured using a FlashEA 1112 Series Elemental Analyzer.

**Statistical Analysis**

Statistical analyses were performed using R software version 3.2.0 (R Development Core Team, 2015). Since each experiment was performed with larvae from a different hatch, each experiment was analyzed separately.

Survival and developmental time of the experiments 1 to 4, as well as DM and CHN of the experiments 1 to 3, were analyzed by one-way ANOVA using temperature, photoperiod, light intensity or light regime as factor. Normality and homogeneity were verified by Shapiro-Wilk and Levene tests. The statistical significance was established with a critical level ($\alpha$) of 0.05 to reject the null hypothesis. Significant treatment level effects after significant ANOVA were evaluated using the post-hoc Tukey-HSD test. The replication unit for the survival was the beaker in which the larvae were reared, the survival data were transformed when required to meet the assumptions of ANOVA using the arcsine squareroot transformation. The replication unit for the developmental time was the average duration per beaker; the lost of a replicate occurred when survival was zero. Developmental time in experiment 3 (light intensity) ended with a single replicate for the treatment of constant darkness, then we proceed to the ANOVA to test for light treatments excluding the treatment of constant darkness. The replication unit for the DM and CHN were each one of the five analysis realized per larval stage and treatment.

To analyze the combined effects of temperature and light regime we used a two-way Type III ANOVA (R package car 2.0-25: (Fox & Weisberg, 2011). The statistical significance was established with a critical level (α) of 0.05 to reject the null hypothesis. Significant treatment level effects after significant ANOVA were evaluated using the post-hoc Tukey-HSD test.

## Results

### Experiment 1: Temperature

The survival was significantly higher at 15 (44.6 ± 5.3 %) and 21 °C (57.1 ± 2.8 %), than at 24 (12.5 ± 6.9 %) and 27 °C (1.7 ± 1.9 %) ($F_{4,15}$ = 26.47, $p < 0.001$; Fig. 1A). None specimen reached the megalopa stage at 30 °C (the last zoea died without moulting at the 7$^{th}$ day). The duration of the zoeal phase significantly increased as temperature decreased ($F_{4,13}$ = 30.2, $p < 0.001$): it was 14.9 ± 0.5 days at 15 °C, 11.6 ± 0.5 days at 18 °C, 9.1 ± 0.5 days at 21 °C, 8.6 ± 1.8 days at 24 °C and 8.5 ± 0.7 days at 27 °C (Fig. 1B). The effects of the temperature on the survival to first juvenile and the duration of the megalopal stage could not be analyzed due to low number of individuals reaching the juvenile stage: 2 juveniles at 15 °C, 1 juvenile at 18 °C, 4 juveniles at 21 °C, and 1 juvenile at 24 °C. The longest duration of development for the megalopa stage was observed at 15 °C (13.5 ± 0.7 days), and the shortest at 24 °C (6 days).

Temperature affected significantly all parameters studied in inter-molt zoea I. Dry mass was lower at 30 °C than from 15 to 24 °C ($F_{5,24}$ = 10.41, $p < 0.001$). The same tendency was observed in C and N content per individual (C: $F_{5,24}$= 12.02, $p < 0.001$; N: $F_{5,24}$= 13.02, $p < 0.001$) and the C and N percentage per individual (C: $F_{5,24}$ = 11.26, $p < 0.001$; N: $F_{5,24}$ = 8.09, $p < 0.001$). The C:N ratio tend to be significantly lower at 30 °C than at a lower temperatures ($F_{5,24}$ = 3.90, $p < 0.01$; Table 1).

Responses of megalopa biomass to temperature resembled those previously described for zoea I. Biomass decreased significantly at the highest temperature analyzed (24 °C; not enough survivors were available at 27 °C for body composition analysis). On the contrary, no significant differences were observed in the range: 15-21 °C. This pattern was found for DM ($F_{3,16} = 8.97$, $p < 0.01$), C and N content per individual (C: $F_{3,16} = 11.27$, $p < 0.001$; N: $F_{3,16} = 15.38$, $p < 0.001$), C and N percentage per individual (C: $F_{3,16} = 10.26$, $p < 0.001$; N: $F_{3,16} = 9.45$, $p < 0.001$). No significant differences on the C:N ratio were found from 15 to 24 °C treatments($F_{3,16} = 2.15$, $p = 0.13$; Table 2).

**Experiment 2: Photoperiod**

Survival from hatching to megalopa was lower at constant light (24 L) as compared to those treatments were larvae were reared under a light: dark cycle (from 8 to 16 L; $F_{4,15} = 5.30$; $p < 0.01$; Fig. 2A). Survival from newly molted megalopa to first juvenile was lower at constant darkness compared to 8 L ($F_{4,15} = 4.09$; $p < 0.05$), but no significant differences were found in the range from 8 to 24 L (Fig. 2B).

The duration of the zoeal phase was longer at constant darkness (8.4 ± 0.5 days) than at 8 L (7.5 ± 0.3 days; $F_{4,15} = 3.07$; $p = 0.049$; Fig. 2C). The duration of the megalopal stage was significantly shorter in the range from 16 to 24 L (6.3 ± 0.5 days; $F_{4,15} = 19.88$; $p < 0.001$; Fig. 2D).

Dry mass of zoea I varied with the photoperiod: it was higher at 8 and 12 L than at 16 and 24 L; the lowest values of DM occurred at constant darkness ($F_{4,20} = 16.41$, $p < 0.001$). The same tendency was observed in C and N content per individual (C: $F_{4,20} = 19.95$, $p < 0.001$; N: $F_{4,20} = 21.15$, $p < 0.001$) and C:N ratio ($F_{4,20} = 11.71$, $p < 0.001$). The lowest C and N percentage per individual occurred at constant darkness (C: $F_{4,19} = 37.78$, $p < 0.001$; N: $F_{4,20} = 17.15$, $p < 0.001$; see Table 3). By contrast, no significant

effects of photoperiod were found on the dry mass and elemental body composition of early post-molt megalopae ($p > 0.05$, Table 4).

**Experiment 3: Light intensity**

Light intensity did not significantly affect survival during the zoeal phase ($F_{3,12} = 2.21$; $p = 0.14$; Fig. 3A) or from megalopae to first juvenile ($F_{3,10} = 2.61$; $p = 0.11$; Fig. 3B). The duration of the zoeal phase was not significantly affected by light intensity (8.0 ± 0.5 days; $F_{3,12} = 3.13$, $p = 0.07$; Fig. 3C). The 0 lux treatment was removed from the statistical analyses because variation was not observed in this treatment. No significant differences were found on the duration of the megalopa stage from 300 lx (4.2 µmoles $m^{-2}$ $s^{-1}$) to 3,000 lx (42 µmoles $m^{-2}$ $s^{-1}$) (6.1± 0.5 days; $F_{2,7} = 4.06$; $p = 0.68$; Fig. 3D).

Dry mass of zoea I varied with the light treatment: it was significantly lower at constant darkness and increased in the presence of light ($F_{3,16} = 7.94$, $p < 0.05$). The same was observed for C and N content per individual (C: $F_{3,16} = 5.89$, $p < 0.01$; N: $F_{3,16} = 4.24$, $p < 0.05$) and C:N ratio ($F_{3,16} = 11.40$, $p < 0.001$). The percent of C and N did not show significant differences among treatments (C: $F_{3,16} = 2.78$, $p = 0.08$; N: $F_{3,16} = 1.58$, $p = 0.23$; Table 5). Dry mass of megalopa did not vary significantly with light intensity ($F_{3,16} = 1.10$, $p = 0.38$), nor did the C or N content per individual (C: $F_{3,16} = 0.57$; $p = 0.65$; N: $F_{3,16} = 0.67$, $p = 0.58$), or the C:N ratio ($F_{3,16} = 0.55$, $p = 0.66$; Table 6). The percent of C and N was significantly higher at 300 lx as compared with the other treatments (C: $F_{3,16} = 11.96$, $p < 0.001$; N: $F_{3,15} = 24.56$, $p < 0.001$).

**Experiment 4: Effect of photo regime on development from megalopa to juvenile**

Survival from early post-molt megalopae to first juvenile was not affected by the light treatment experienced during the zoeal or the megalopal stage ($F_{3,20} = 0.26$, $p = 0.85$; Fig. 4A). The duration of the megalopal stage varied significantly depending on the

light regime experienced during the megalopal stage and previous larval stages ($F_{3,19}$ = 63.38, $p < 0.001$; Fig. 4B). The longest duration of the megalopal stage occurred when they were reared at constant darkness (average 9.2 ± 0.2 days), independently of the light conditions during the previous zoeal phase. The shortest duration (6.2 ± 0.3 days) of the megalopal stage occurred when larvae were exposed to a light: dark cycle during the whole larval development.

**Experiment 5: Combined effects of light regime and temperature**

There was no effect of temperature (T), light intensity (L) or their interaction (TxL) on survival from hatching to megalopa (T: $F_{1,12} = 1.12$, $p = 0.30$; L: $F_{1,12} = 0.30$, $p = 0.60$; TxL: $F_{1,12} = 4.36$, $p = 0.06$; Fig. 5A). The effect of temperature was significant on the survival from megalopa to first juvenile stage, where survival was higher at 21 ºC than at 18 °C, but no significant effect was observed with the light treatment or interacting with temperature (T: $F_{1,9} = 6.6$, $p < 0.05$; L: $F_{1,9} = 1.4$, $p = 0.27$; TxL: $F_{1,9} = 1.1$, $p = 0.33$; Fig. 5B).

The duration of the zoeal development was affected by temperature but not by light treatment, either as a main factor on in interaction with temperature (T: $F_{1,10} = 92.56$, $p < 0.001$; L: $F_{1,10} = 3.13$, $p = 0.11$; TxL: $F_{1,10} = 0.20$, $p = 0.66$). The zoeal phase was shorter at 21 °C (8.8 ± 0.6 days) than at 18 °C (14.3 ± 1.5 days; Fig. 5C). The duration of the megalopal stage could not be tested due to low number of individuals reaching the juvenile stage. On average the duration of the megalopa kept at 21 °C at constant darkness required 8.3 ±2.3 days, and at light: dark cycle 6.0 ± 0.2 days (Fig. 5D). At 18 °C only one individual reached the juvenile stage at constant darkness (on day 13$^{th}$) and three under a light: dark cycle (average = 9 ± 1 days).

**Discussion**

The optimal temperature for the larval culture of *M. brachydactyla* is 21 ± 1 °C: a lower temperature increases the duration of larval development without enhance significantly the survival, while a higher temperature reduces survival without shortening the duration of the larval development. The larvae of *M. brachydactyla* in captivity can tolerate a temperature range similar to the measured in the coastal waters of the NW Iberian Peninsula (Gago, Cabanas, Casas & Miranda, 2011). Similar trends occur in other species that inhabit cold to temperate waters (e.g. *Hyas araneus*: 6-12 °C, Anger, 1983; *Metacarcinus magister*: ~10 °C, Reed, 1969; Sulkin & McKeen, 1989; *Cancer irroratus*: 15-24 °C, Johns, 1981). By contrast, the species that inhabit tropical to warm temperate waters have an increased range for suitable temperatures (e.g. *Stenorhynchus seticornis*: 25-28 °C, Hernández, Palazón-Fernández, Hernández & Bolaños, 2012; *Mithraculus forceps* and *M. sculptus*: 25-28 °C, Penha-Lopes, Rhyne, Lin & Narciso, 2005; Rhyne, Penha-Lopes & Lin, 2005; *Scylla serrata*: 25-30 °C, Hamasaki, 2003).

The present study shows that a light: dark cycle is more suitable for the larval culture of *M. brachydactyla*, independently of the duration of the light phase, as observed during the larval culture of other brachyurans, e.g., *Ranina ranina* (Minagawa, 1994), *Carcinus maenas* (Dawirs, 1982), *Portunus pelagicus* (Andrés et al., 2010) and *Ucides cordatus* (Cottens, Silva, Ventura, Ramos & Ostrensky, 2014). In brachyurans as *Sesarma reticulatum* the survival of the larvae decreases with the shortening of the light period (Costlow & Bookhout, 1962),while in the anomurans as *Birgus latro* the survival of the larvae decreases when cultured in constant darkness (Hamasaki, Ogiso, Dan and Kitada, 2016); but such influence is not clear in the present study. The survival rate of *M. brachydactyla* larvae did not appear to be influenced by light intensity in the range 300-3000 lx (ca. 4.2 - 42 µmoles $m^{-2}$ $s^{-1}$). Similar situation was observed in *U. cordatus* in the range from 210 to 710 lx (Cottens et al., 2014).

334 The duration of the megalopal stage of *M. brachydactyla* shortenedin presence of light,
335 as observed in *C. maenas* (Dawirs, 1982). However, the megalopae of *M. brachydactyla*
336 required longer to reach the first juvenile stage when reared under constant darkness
337 during the zoeal phase. The influence of the culture conditions during previous stages
338 over the megalopa seems to be a latent effect (Pechenik, 2006); i.e., environmental
339 conditions experienced at early stages affects development or other traits at later stages.
340 Latent effects have been reported in decapod crustaceans in response to food limitation
341 or osmotic stress (Giménez, 2006; Torres, Giménez & Anger, 2008; Giménez, 2010).
342 The zoea I of *M. brachydactyla* reared at constant darkness had lower dry mass, carbon
343 and nitrogen content per individual, and C:N ratio than larvae reared in the presence of
344 light; but in general (excepting for 300 lx treatment) no variations were observed among
345 treatments when the larvae reached the megalopa stage. It is likely that lower larval
346 quality caused by constant darkness led to compensatory effects at the megalopa stage,
347 which may have also involved delaying the time of metamorphosis (Figs. 2D; 3D).

348 The mechanisms in which the light influences the larval development are not well
349 known. The zoeae of *M. brachydactyla* reared in presence of light (independently of the
350 light source) showed higher DM, carbon and nitrogen content, carbon and nitrogen
351 percentage, and C/N ratio than zoeae reared in constant darkness. Since brachyuran
352 larvae are non-obligate visual feeders and can complete the larval development in
353 absence of light (Costlow & Bookhout, 1962; Cronin & Forward Jr, 1980; Dawirs,
354 1982; Minagawa, 1994; Gardner & Maguire, 1998; Andrés et al., 2010), has been
355 proposed that light stimulates swimming activity (Forward Jr, 1974; Forward Jr &
356 Cronin, 1980; Cronin & Forward Jr, 1980), increasing the chances of prey encounter,
357 and consequently the food intake in several brachyuran species (Andrés et al., 2010;
358 Rabbani & Zeng, 2005; Gardner & Maguire, 1998; Minagawa, 1994; Cronin &

359 Forward Jr, 1980). Other hypothesis proposes that light influences the efficiency of the
360 food conversion: the shrimp *Penaeus merguiensis* showed better food conversion
361 efficiency at mid light intensities (750 lx) than at low light intensities (75 lx) (Hoang et
362 al., 2003), while the congeneric *Penaeus chinensis* showed better efficiency at low-mid
363 light intensities (50 - 1,300 lx) than at high light intensities (5,500 lx) (Wang et al.,
364 2004). The authors proposed that high light intensities stimulate respiration and
365 excretion; therefore, less energy is retained for growth. In this sense, the megalopa of *M.*
366 *brachydactyla* exposed at 300 lx showed higher percentage of carbon and nitrogen in
367 comparison to higher light intensities or constant darkness.

368 In summary, the optimal temperature for rearing larvae of *M. brachydactyla* is ~21 °C
369 and temperature shows stronger influence than light over the larval development. It is
370 recommended to maintain a light: dark regime since in presence of light the duration of
371 the megalopa stage is shortened. The maintenance of a light: dark cycle regime was also
372 recommended for the larval culture of other brachyuran species (Minagawa, 1994;
373 Gardner & Maguire, 1998). In culture conditions, the larval development of *M.*
374 *brachydactyla* requires in average 31 days at 14 - 17 ºC (Pazos et al., 2018 ). In our
375 optimized culture conditions, we are able to reduce the time of development to
376 approximately 14 days.

377 **Acknowledgements**

378 The Spanish Ministry of Economy and Competitiveness funded bench fees
379 through the INIA project [grant number RTA2011-00004-00-00] to G.G and a
380 predoctoral fellowship to D.C. [FPI-INIA]. The authors would like to thank Karl B.
381 Andree for their comments that improved this manuscript. We thank hatchery
382 technicians at IRTA in Sant Carles de la Ràpita (David Carmona, Glòria Macià, Magda

Monllaó and Francesc X. Ingla) for their help in the broodstock maintenance and setup of the rearing culture system.

## References

Andrés M., Estévez A., Anger K., Rotllant G. (2008) Developmental patterns of larval growth in the edible spider crab, *Maja brachydactyla* (Decapoda: Majidae). *Journal of Experimental Marine Biology and Ecology,* **357**, 35-40.

Andrés M., Rotllant G., Zeng C. (2010) Survival, development and growth of larvae of the blue swimmer crab, *Portunus pelagicus*, cultured under different photoperiod conditions. *Aquaculture,* **300**, 218-222.

Anger K. (1983) Temperature and the larval development of *Hyas araneus* L. (Decapoda : Majidae); extrapolation of laboratory data to field conditions. *Journal of Experimental Marine Biology and Ecology,* **69**, 203-215.

Anger K. (2001) *The Biology of Decapod Crustacean Larvae,* A.A. Balkema Publishers, Rotterdam.

Anger K., Harms J. (1990) Elemental (CHN) and proximate biochemical composition of decapod crustacean larvae. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,* **97B**, 69-80.

Boeuf G., Le Bail P.-Y. (1999) Does light have an influence on fish growth? *Aquaculture,* **177**, 129-152.

Castejón D., Rotllant G., Giménez L., Torres G., Guerao G. (2015) The effects of temperature and salinity on the survival, growth and duration of the larval

development of the common spider crab *Maja brachydactyla* (Balss, 1922) (Brachyura: Majidae). *Journal of Shellfish Research,* **34**, 1073-1083.

Clark P.F. (1986) The larval stages of *Maja squinado* (Herbst, 1788) (Crustacea: Brachyura: Majidae) reared in the laboratory. *Journal of Natural History,* **20**, 825-836.

Costlow J.D., Bookhout C.G. (1962) The larval development of *Sesarma reticulatum* Say- Reared in the Laboratory. *Crustaceana,* **4**, 281-294.

Cottens K.F., Silva U.A.T., Ventura R., Ramos F.M., Ostrensky A. (2014) Cultivo de larvas de *Ucides cordatus* (LINNAEU, 1763) sob diferentes intensidades luminosas. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* **66**, 1464-1470.

Cronin T.W., Forward Jr R.B. (1980) The Effects of Starvation on Phototaxis and Swimming of Larvae of the Crab *Rhithropanopeus harrisi*. *Biological Bulletin,* **158**, 283-294.

Dawirs R.R. (1982) Methodical aspects of rearing decapod larvae, *Pagurus bernhardus* (Paguridae) and *Carcinus maenas* (Portunidae). *Helgoländer Meeresuntersuchungen,* **35**, 439-464.

Epifanio C.E., Cohen J.H. (2016) Behavioral adaptations in larvae of brachyuran crabs: A review. *Journal of Experimental Marine Biology and Ecology,* **482**, 85-105.

FAO (2014) B-42 Crabs, sea-spiders. Capture production by species, fishing areas and countries or areas. In: *FAO yearbook. Fishery and aquaculture statistics* (ed by FAO). FAO, Rome.

Forward Jr R.B. (1974) Negative phototaxis in crustacean larvae: Possible functional significance. *Journal of Experimental Marine Biology and Ecology,* **16**, 11-17.

Forward Jr R.B., Cronin T.W. (1980) Tidal Rhythms of Activity and Phototaxis of an Stuarine Crab Larva. *Biological Bulletin,* **158**, 295-303.

Fox J., Weisberg S. (2011) An {R} Companion to Applied Regression. Sage, Second Edition, Thousand Oaks CA.

Gago J., Cabanas J.M., Casas G., Miranda A. (2011) Thermohaline measurements in the continental shelf zone of the NW Iberian Peninsula, 1994–2006. *Climate Research,* **48**, 219-229.

Gardner C., Maguire G.B. (1998) Effect of photoperiod and light intensity on survival, development and cannibalism of larvae of the Australian giant crab *Pseudocarcinus gigas* (Lamarck). *Aquaculture,* **165**, 51-63.

Giménez L. (2006) Phenotypic links in complex life cycles: conclusions from studies with decapod crustaceans. *Integrative and Comparative Biology,* **46**, 615-622.

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

González-Gurriarán E., Fernández L., Freire J., Muiño R. (1998) Mating and role of seminal receptacles in the reproductive biology of the spider crab *Maja squinado* (Decapoda, Majidae). *Journal of Experimental Marine Biology and Ecology,* **220**, 269-285.

Guerao G., Pastor E., Martin J., Andrés M., Estévez A., Grau A., Duran J., Rotllant G. (2008) The larval development of *Maja squinado* and *M. brachydactyla* (Decapoda, Brachyura, Majidae) described from plankton collected and laboratory-reared material. *Journal of Natural History,* **42**, 2257-2276.

Hamasaki K. (2003) Effects of temperature on the egg incubation period, survival and developmental period of larvae of the mud crab *Scylla serrata* (Forskål) (Brachyura: Portunidae) reared in the laboratory. *Aquaculture,* **219**, 561-572.

Hamasaki K., Ogiso Y., Dan S., Kitada S. (2016) Survival, development and growth of larvae of the coconut crab, *Birgus latro*, cultured under different photoperiod conditions. *Aquaculture Research,* **47**, 2506-2517.

Hernández J.E., Palazón-Fernández J.L., Hernández G., Bolaños J. (2012) The effect of temperature and salinity on the larval development of *Stenorhynchus seticornis* (Brachyura: Inachidae) reared in the laboratory. *Journal of the Marine Biological Association of the United Kingdom,* **92**, 505-513.

Hoang T., Barchiesis M., Lee S.Y., Keenan C.P., Marsden G.E. (2003) Influences of light intensity and photoperiod on moulting and growth of *Penaeus merguiensis* cultured under laboratory conditions. *Aquaculture,* **216**, 343-354.

463 Johns D.M. (1981) Physiological studies on *Cancer irroratus* larvae. I. Effects of
464 temperature and salinity on survival, development rate and size. *Marine Ecology*
465 *Progress Series,* **5**, 75-83.

466 Minagawa M. (1994) Effects of photoperiod on survival, feeding and development of
467 larvae of the red frog crab, *Ranina ranina*. *Aquaculture,* **120**, 105-114.

468 Pazos G., Fernández J., Linares F., Sánchez J., Otero J.J., Iglesias J., Domingues P.
469 (2018) The complete life cycle in captivity of the spider crab, *Maja*
470 *brachydactyla*, Herbst 1788. *Aquaculture Research,* **0**.

471 Pechenik J.A. (2006) Larval experience and latent effects-metamorphosis is not a new
472 beginning. *Integrative and Comparative Biology,* **46**, 323-333.

473 Penha-Lopes G., Rhyne A.L., Lin J., Narciso L. (2005) The larval rearing of the marine
474 ornamental crab, *Mithraculus forceps* (A. Milne Edwards, 1875) (Decapoda:
475 Brachyura: Majidae). *Aquaculture Research,* **36**, 1313-1321.

476 R Development Core Team (2015) R: A language and environment for statistical
477 computing. R Foundation for Statistical Computing, Vienna, Austria.

478 Rabbani A.G., Zeng C. (2005) Effects of tank colour on larval survival and
479 development of mud crab *Scylla serrata* (Forskål). *Aquaculture Research,* **36**,
480 1112-1119.

481 Reed P.H. (1969) Culture Methods and Effects of Temperature and Salinity on Survival
482 and Growth of Dungeness Crab (*Cancer magister*) Larvae in the Laboratory.
483 *Journal of the Fisheries Research Board of Canada,* **26**, 389-397.

Rhyne A.L., Penha-Lopes G., Lin J. (2005) Growth, development, and survival of larval *Mithraculus sculptus* (Lamark) and *Mithraculus forceps* (A. Milne Edwards) (Decapoda: Brachyura: Majidae): economically important marine ornamental crabs. *Aquaculture,* **245**, 183-191.

Rotllant G., Simeó C.G., Macià G., Estévez A. (2015) High environmental salinity reduces the reproductive potential of the spider crab *Maja brachydactyla* (Decapoda, Majidae). *Marine Ecology,* **36**, 496-505.

Sulkin S.D., McKeen G.L. (1989) Laboratory study of survival and duration of individual zoeal stages as a function of temperature in the brachyuran crab *Cancer magister*. *Marine Biology,* **103**, 31-37.

Thorson G. (1964) Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. *Ophelia,* **1**, 167-208.

Torres G., Giménez L., Anger K. (2008) Cumulative effects of low salinity on larval growth and biochemical composition in an estuarine crab, *Neohelice granulata*. *Aquatic Biology,* **2**, 37-45.

Ulikowski D., Krzywosz T. (2004) The impact of photoperiod and stocking density on the growth and survival of narrow-clawed crayfish (*Astacus leptodactylus* Esch.) larvae. *Archives of Polish Fisheries,* **12**, 81-86.

Verísimo P., Bernárdez C., González-Gurriarán E., Freire J., Muino R., Fernández L. (2011) Changes between consecutive broods in the fecundity of the spider crab, *Maja brachydactyla*. *Ices Journal of Marine Science,* **68**, 472-478.

505 Viherluoto M., Viitasalo M. (2001) Effect of light on the feeding rates of pelagic and
506 littoral mysid shrimps: a trade-off between feeding success and predation
507 avoidance. *Journal of Experimental Marine Biology and Ecology,* **261**, 237-244.

508 Villamizar N., Blanco-Vives B., Migaud H., Davie A., Carboni S., Sánchez-Vázquez
509 F.J. (2011) Effects of light during early larval development of some
510 aquacultured teleosts: A review. *Aquaculture,* **315**, 86-94.

511 Wang F., Dong S., Dong S., Huang G., Zhu C., Mu Y. (2004) The effect of light
512 intensity on the growth of Chinese shrimp *Fenneropenaeus chinensis*.
513 *Aquaculture,* **234**, 475-483.

514 Wickins J.F., O'C Lee D. (2002) *Crustacean Farming, Ranching and Culture, 2nd*
515 *edition,* Wiley-Blackwell, Oxford.

516

517

518 **Figure legends**

519 **Figure 1**. *Maja brachydactyla*. Influence of temperature on survival and
520 duration of the zoeal phase. A. Survival from hatching to megalopa stage (n = 4 per
521 treatment). B. Duration of the zoeal phase (*n* = 4 per treatment). Bars show average ±
522 SD. Different letters indicate significant differences ($p < 0.05$; post-hoc Tukey HSD).

523 **Figure 2**. *Maja brachydactyla*. Influence of photoperiod (L, light hours) on
524 survival and duration of the larval development. A. Survival from hatching to megalopa
525 stage (*n* = 4 per treatment). B. Survival from megalopa to first juvenile stage (*n* = 4 per
526 treatment). C. Duration of the zoeal phase (*n* = 4 per treatment). D. Duration of the
527 megalopal stage (*n* = 4 per treatment). Bars show average ± SD. Different letters
528 indicate significant differences ($p < 0.05$; post-hoc Tukey HSD).

529 **Figure 3**. *Maja brachydactyla*. Influence of light intensity (lx, lux) on survival
530 and duration of the larval development. A. Survival from hatching to megalopa stage (*n*
531 = 4 per treatment). B. Survival from megalopa to first juvenile (*n* = 2, 4, 4, 4,
532 respectively). C. Duration of the zoeal phase (*n* = 4 per treatment). D. Duration of the
533 megalopal stage (*n* = 1, 4, 4, 4, respectively). Bars show average ± SD. Different letters
534 show significant differences ($p < 0.05$; post-hoc Tukey HSD).

535 **Figure 4**. *Maja brachydactyla*. Influence of photoperiod on metamorphosis
536 from megalopa to first juvenile. A. Survival from megalopa to first juvenile stage (*n* = 6
537 per treatment). B. Duration of the megalopal stage (*n* = 6 per treatment). Treatments: D
538 = constant darkness (0 light hours). L = light-dark cycle (12 h light: 12 h dark). First
539 letter: treatment during the zoeal phase. Second letter: treatment during the megalopal
540 stage. Bars show average ± SD. Different letters indicate significant differences ($p <$
541 0.05; post-hoc Tukey HSD).

542 **Figure 5**. *Maja brachydactyla*. Influence of combined light regime and
543 temperature on survival and duration of the larval development. Treatments: D =
544 constant darkness (0 light hours). L = light-dark cycle (12 h light: 12 h dark). A.
545 Survival from hatching to megalopal stage (*n* = 4 per treatment). B. Survival from
546 megalopa to first juvenile stage (*n* = 4, 2, 3, 4, respectively). C. Duration of the zoeal
547 phase (*n* = 4, 2, 4, 4, respectively). D. Duration of the megalopal stage (no statistical

548 analysis available; $n$ = 1, 1, 3, 4, respectively). Bars show average ± SD. Different
549 letters show significant differences ($p < 0.05$; post-hoc Tukey HSD).

550 **Supplementary Figure 1**. Emission spectra of the light sources employed in the
551 experiments, provided by the manufacturer. Continuous line: fluorescent tubes. Dotted
552 line: LED lights.

553

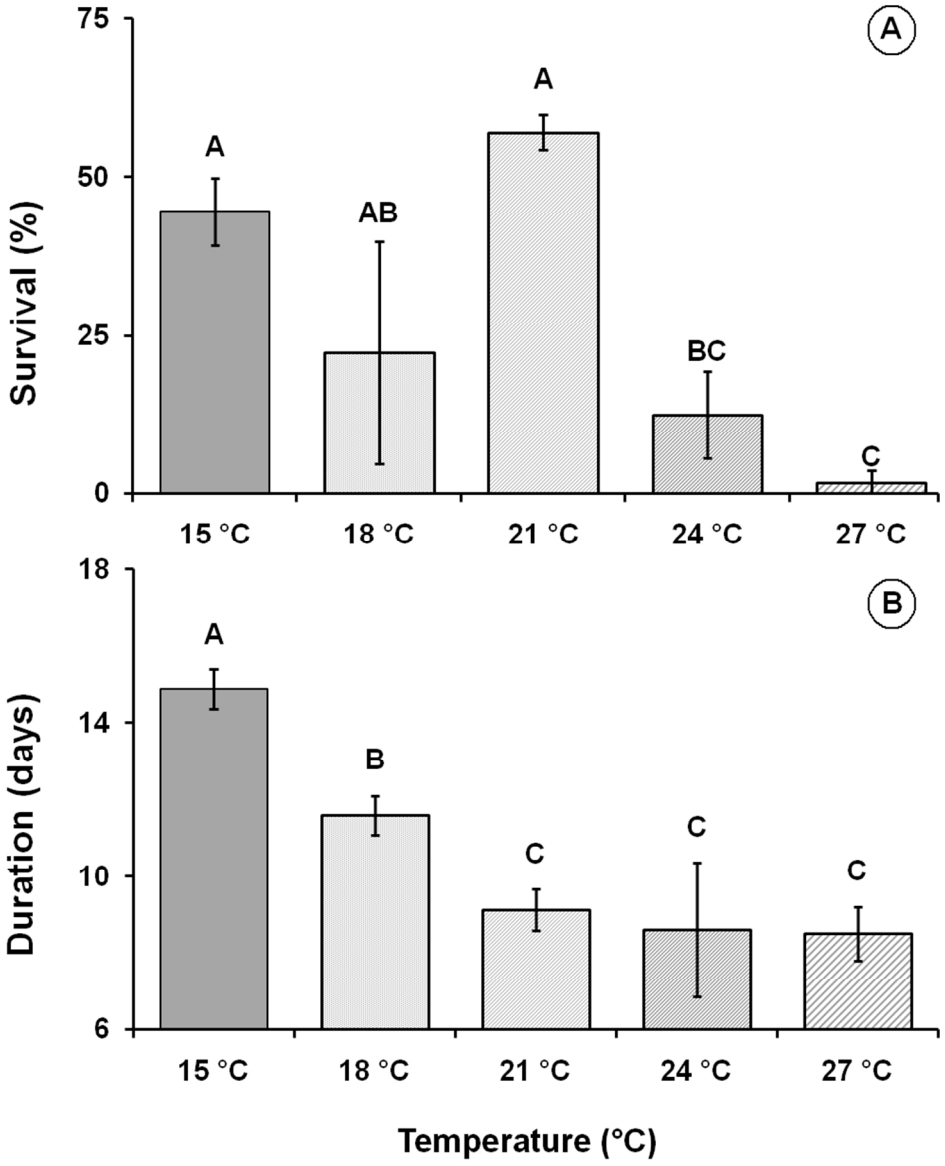



**Figure 1**. *Maja brachydactyla*. Influence of temperature on survival and duration of the zoeal phase. A. Survival from hatching to megalopa stage (n = 4 per treatment). B. Duration of the zoeal phase (n = 4 per treatment). Bars show average ± SD. Different letters indicate significant differences ($p < 0.05$; post-hoc Tukey HSD).

270x335mm (96 x 96 DPI)

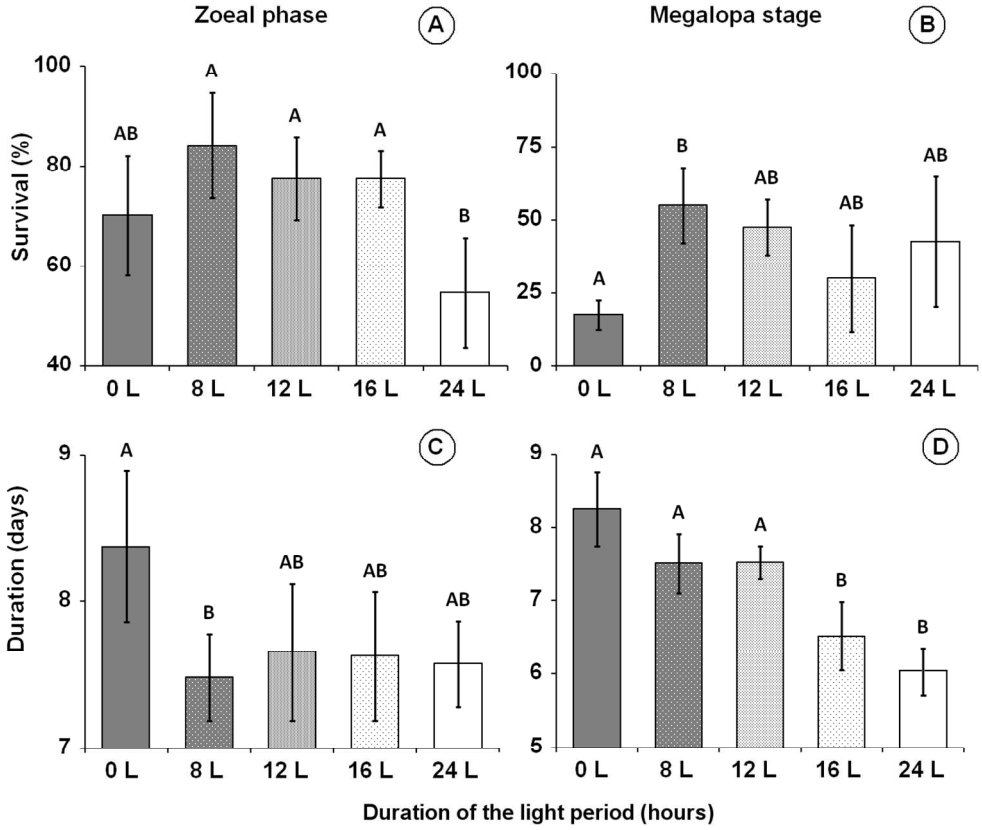



**Figure 2**. *Maja brachydactyla*. Influence of photoperiod (L, light hours) on survival and duration of the larval development. A. Survival from hatching to megalopa stage (n = 4 per treatment). B. Survival from megalopa to first juvenile stage (n = 4 per treatment). C. Duration of the zoeal phase (n = 4 per treatment). D. Duration of the megalopal stage (n = 4 per treatment). Bars show average ± SD. Different letters indicate significant differences ($p < 0.05$; post-hoc Tukey HSD).

371x321mm (96 x 96 DPI)

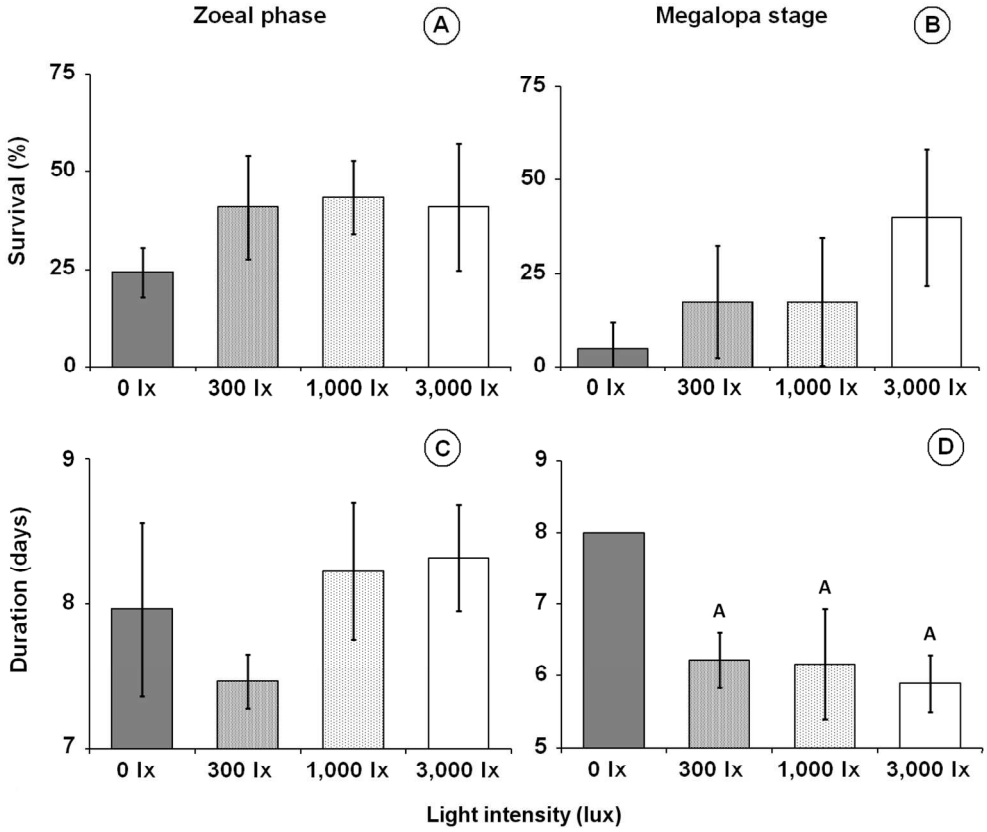



**Figure 3**. *Maja brachydactyla*. Influence of light intensity (lx, lux) on survival and duration of the larval development. A. Survival from hatching to megalopa stage (n = 4 per treatment). B. Survival from megalopa to first juvenile (n = 2, 4, 4, 4, respectively). C. Duration of the zoeal phase (n = 4 per treatment). D. Duration of the megalopal stage (n = 1, 4, 4, 4, respectively). Bars show average ± SD. Different letters show significant differences ($p < 0.05$; post-hoc Tukey HSD).

371x328mm (96 x 96 DPI)

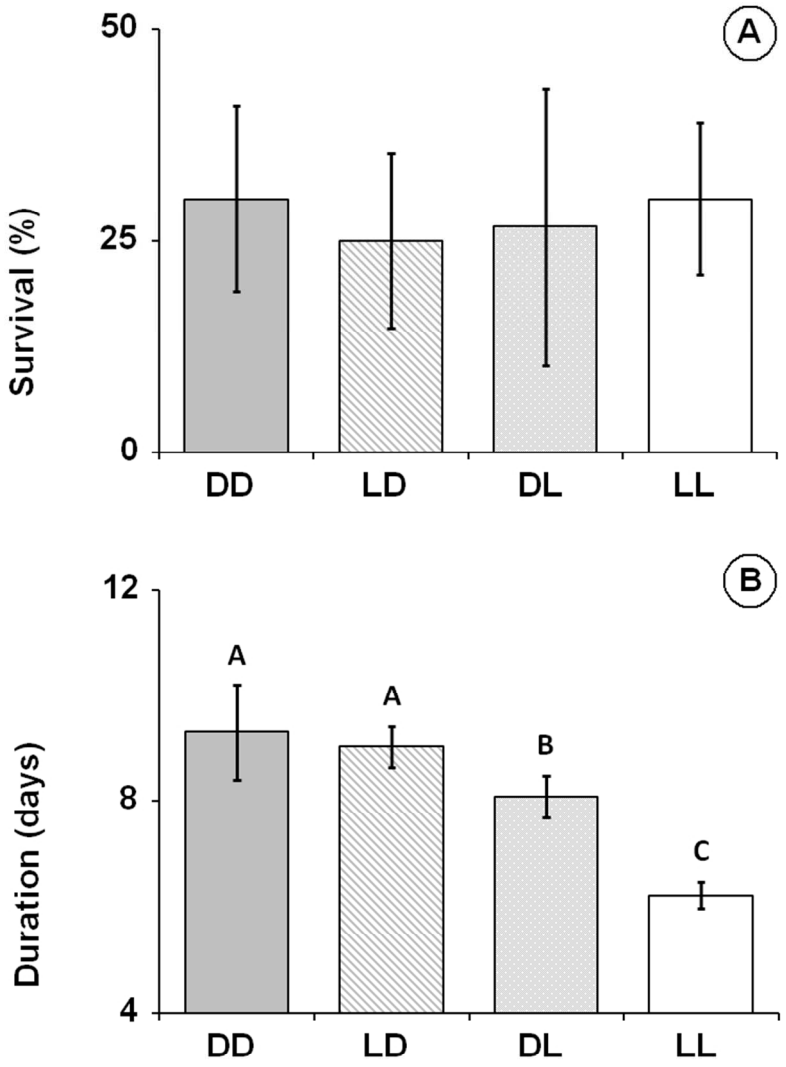



**Figure 4**. *Maja brachydactyla*. Influence of photoperiod on metamorphosis from megalopa to first juvenile. A. Survival from megalopa to first juvenile stage (n = 6 per treatment). B. Duration of the megalopal stage (n = 6 per treatment). Treatments: D = constant darkness (0 light hours). L = light-dark cycle (12 h light: 12 h dark). First letter: treatment during the zoeal phase. Second letter: treatment during the megalopal stage. Bars show average ± SD. Different letters indicate significant differences ($p < 0.05$; post-hoc Tukey HSD).

213x302mm (96 x 96 DPI)

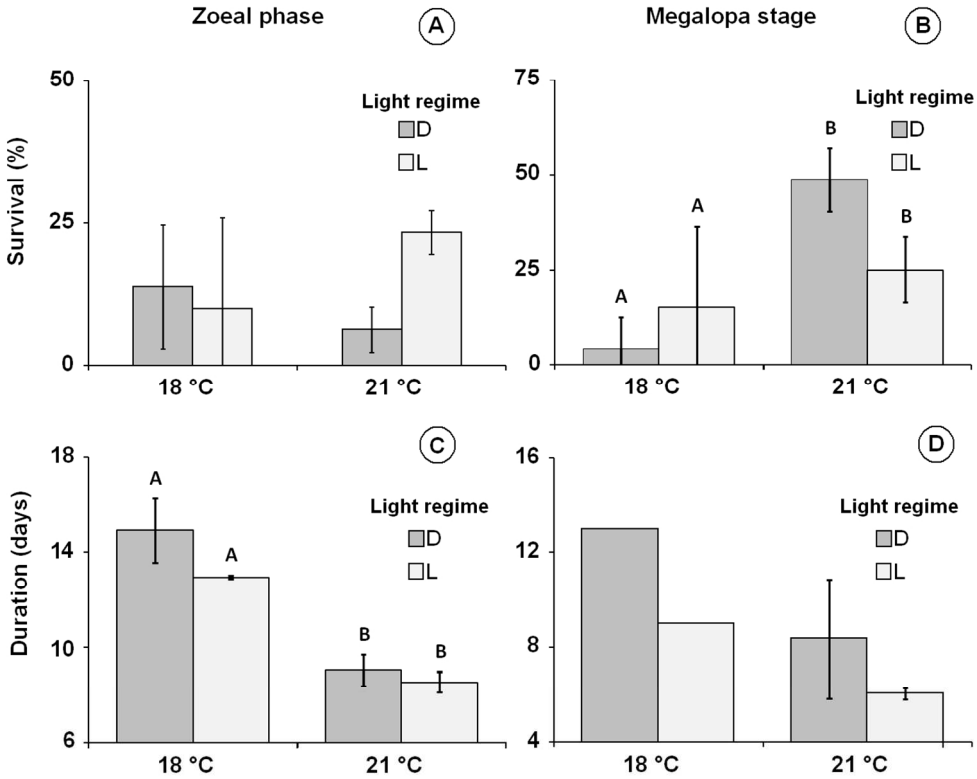
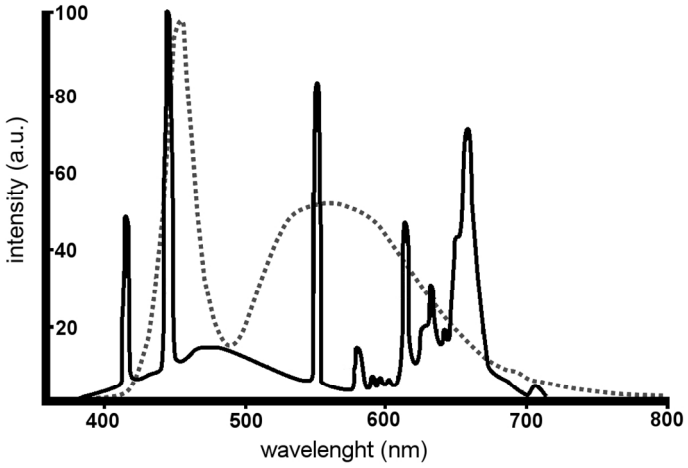



**Figure 5**. *Maja brachydactyla*. Influence of combined light regime and temperature on survival and duration of the larval development. Treatments: D = constant darkness (0 light hours). L = light-dark cycle (12 h light: 12 h dark). A. Survival from hatching to megalopal stage (n = 4 per treatment). B. Survival from megalopa to first juvenile stage (n = 4, 2, 3, 4, respectively). C. Duration of the zoeal phase (n = 4, 2, 4, 4, respectively). D. Duration of the megalopal stage (no statistical analysis available; n = 1, 1, 3, 4, respectively). Bars show average ± SD. Different letters show significant differences ($p < 0.05$; post-hoc Tukey HSD).

374x302mm (96 x 96 DPI)




**Supplementary Figure 1**. Emission spectra of the light sources employed in the experiments, provided by the manufacturer. Continuous line: fluorescent tubes. Dotted line: LED lights.

532x303mm (72 x 72 DPI)

**Table 1**. *Maja brachydactyla*. Influence of the temperature (°C) on the dry mass and elemental body composition of zoeae I [newly hatched zoea (0 days) to intermoult (2 days)]. Dry mass (DM), carbon content per individual (C), carbon percentage (C %), nitrogen content per individual (N), nitrogen percentage (N %) and C:N ratio. Values are expresed as average ± SD.

| Age (days) | Temp (± 1 °C) | DM (µg) | Carbon (µg ind$^{-1}$) | Carbon (%) | Nitrogen (µg ind$^{-1}$) | Nitrogen (%) | C:N ratio |
|---|---|---|---|---|---|---|---|
| 0 | - | 99 ± 5 | 32.0 ± 0.4 | 32.5 ± 1.2 | 8.08 ± 0.09 | 8.20 ± 0.35 | 3.96 ± 0.03 |
| 2 | 15 | 114 ± 3$^{ab}$ | 35.0 ± 1.6$^{ab}$ | 30.6 ± 0.6$^{ac}$ | 8.72 ± 0.37$^{ab}$ | 7.64 ± 0.16$^{ac}$ | 4.01 ± 0.04$^{ab}$ |
| | 18 | 119 ± 8$^{ab}$ | 38.5 ± 3.8$^{a}$ | 32.2 ± 1.1$^{ab}$ | 9.39 ± 0.80$^{a}$ | 7.87 ± 0.15$^{ab}$ | 4.09 ± 0.06$^{a}$ |
| | 21 | 116 ± 4$^{ab}$ | 36.4 ± 2.4$^{ab}$ | 31.2 ± 1.0$^{abc}$ | 9.03 ± 0.41$^{ab}$ | 7.75 ± 0.12$^{ab}$ | 4.03 ± 0.09$^{ab}$ |
| | 24 | 123 ± 5$^{a}$ | 39.8 ± 2.7$^{a}$ | 32.4 ± 1.3$^{b}$ | 9.73 ± 0.56$^{a}$ | 7.94 ± 0.32$^{a}$ | 4.08 ± 0.08$^{a}$ |
| | 27 | 110 ± 8$^{bc}$ | 33.2 ± 3.1$^{b}$ | 30.2 ± 0.7$^{cd}$ | 8.32 ± 0.67$^{bc}$ | 7.59 ± 0.07$^{bc}$ | 3.99 ± 0.05$^{ab}$ |
| | 30 | 99 ± 1$^{c}$ | 28.6 ± 0.8$^{c}$ | 28.8 ± 0.4$^{d}$ | 7.27 ± 0.21$^{c}$ | 7.31 ± 0.13$^{c}$ | 3.94 ± 0.05$^{b}$ |

Different letters indicate significant differences ($P < 0.05$) among treatments.

**Table 2**. *Maja brachydactyla*. Influence of the temperature (°C) on the dry mass and elemental body composition of newly molted megalopae. Dry mass (DM), carbon content per individual (C), carbon percentage (C%), nitrogen content per individual (N), nitrogen percentage (N%) and C:N ratio. Values are expresed as average ± SD.

| Temperature (± 1 °C) | DM (µg) | Carbon (µg ind$^{-1}$) | Carbon (%) | Nitrogen (µg ind$^{-1}$) | Nitrogen (%) | C:N ratio |
|---|---|---|---|---|---|---|
| 15 | 205 ± 10$^{a}$ | 75.0 ± 4.3$^{a}$ | 36.6 ± 0.9$^{a}$ | 17.83 ± 0.63$^{a}$ | 8.71 ± 0.29$^{a}$ | 4.21 ± 0.12 |
| 18 | 200 ± 11$^{a}$ | 69.7 ± 6.0$^{a}$ | 34.8 ± 1.1$^{ab}$ | 17.10 ± 0.84$^{a}$ | 8.55 ± 0.21$^{ab}$ | 4.07 ± 0.17 |
| 21 | 193 ± 18$^{ab}$ | 65.9 ± 9.3$^{a}$ | 34.0 ± 2.2$^{bc}$ | 15.87 ± 1.82$^{a}$ | 8.20 ± 0.32$^{bc}$ | 4.14 ± 0.13 |
| 24 | 164 ± 13$^{b}$ | 52.4 ± 5.0$^{b}$ | 31.9 ± 0.9$^{c}$ | 13.04 ± 1.17$^{b}$ | 7.92 ± 0.18$^{c}$ | 4.02 ± 0.04 |

Different letters indicate significant differences ($P < 0.05$) among treatments.

**Table 3**. *Maja brachydactyla*. Influence of the photoperiod (L, light hours) on the dry mass and elemental body composition of zoeae I [newly hatched zoea (0 days) to intermoult (2 days)] reared at 21 °C. Dry mass (DM), carbon content per individual (C), carbon percentage (C %), nitrogen content per individual (N), nitrogen percentage (N %) and C:N ratio. Values are expresed as average ± SD.

| Age (days) | Photoperiod (light hours) | DM (µg) | Carbon (µg/ind) | Carbon (%) | Nitrogen (µg/ind) | Nitrogen (%) | C:N ratio |
|---|---|---|---|---|---|---|---|
| 0 | - | 118 ± 1 | 36.4 ± 0.5 | 30.8 ± 0.3 | 9.16 ± 0.07 | 7.74 ± 0.08 | 3.98 ± 0.03 |
| 2 | 0 L | 122 ± 5$^{a}$ | 36.5 ± 2.0$^{a}$ | 30.0 ± 0.7$^{a}$ | 9.18 ± 0.50$^{a}$ | 7.54 ± 0.18$^{a}$ | 3.97 ± 0.03$^{a}$ |
| | 8 L | 150 ± 4$^{b}$ | 50.8 ± 2.1$^{bc}$ | 33.9 ± 0.5$^{b}$ | 12.11 ± 0.39$^{bc}$ | 8.09 ± 0.06$^{b}$ | 4.20 ± 0.04$^{bc}$ |
| | 12 L | 149 ± 4$^{b}$ | 52.6 ± 1.5$^{b}$ | 35.4 ± 0.3$^{c}$ | 12.41 ± 0.27$^{b}$ | 8.34 ± 0.12$^{b}$ | 4.24 ± 0.06$^{c}$ |
| | 16 L | 135 ± 8$^{c}$ | 45.5 ± 4.1$^{c}$ | 33.5 ± 1.3$^{bd}$ | 11.10 ± 0.78$^{c}$ | 8.20 ± 0.15$^{b}$ | 4.09 ± 0.08$^{ab}$ |
| | 24 L | 138 ± 9$^{bc}$ | 45.5 ± 4.7$^{c}$ | 32.3 ± 1.4$^{d}$ | 11.14 ± 0.90$^{c}$ | 8.05 ± 0.24$^{b}$ | 4.08 ± 0.10$^{ab}$ |

Different letters indicate significant difference ($p < 0.05$) among treatments.

**Table 4**. *Maja brachydactyla*. Influence of the photoperiod (L, light hours) on the dry mass and elemental body composition of newly molted megalopae reared at 21 °C. Dry mass (DM), carbon content per individual (C), carbon percentage (C%), nitrogen content per individual (N), nitrogen percentage (N%) and C:N ratio. Values are expresed as average ± SD.

| Photoperiod (light hours) | DM (µg) | Carbon (µg/ind) | Carbon (%) | Nitrogen (µg/ind) | Nitrogen (%) | C:N ratio |
|---|---|---|---|---|---|---|
| 0 L | 189 ± 18 | 62.3 ± 2.6 | 33.1 ± 0.5 | 15.99 ± 0.52 | 8.50 ± 0.26 | 3.89 ± 0.08 |
| 8 L | 201 ± 07 | 66.5 ± 1.2 | 33.0 ± 0.9 | 16.67 ± 0.15 | 8.28 ± 0.29 | 3.99 ± 0.09 |
| 12 L | 206 ± 21 | 67.0 ± 3.1 | 32.5 ± 0.8 | 16.70 ± 0.66 | 8.11 ± 0.24 | 4.01 ± 0.09 |
| 16 L | 201 ± 12 | 67.2 ± 3.4 | 33.4 ± 2.7 | 16.84 ± 0.75 | 8.37 ± 0.63 | 3.99 ± 0.07 |
| 24 L | 196 ± 16 | 65.6 ± 2.2 | 33.6 ± 1.8 | 16.47 ± 0.42 | 8.44 ± 0.37 | 3.98 ± 0.08 |

Significant differences among treatments were not observed.

**Table 5**. *Maja brachydactyla*. Influence of the light intensity (lux) on the dry mass and elemental body composition of zoeae I [newly hatched zoea (0 days) to intermoult (2 days)] reared at 21 °C. Dry mass (DM), carbon content per individual (C), carbon percentage (C %), nitrogen content per individual (N), nitrogen percentage (N %) and C:N ratio. Values are expresed as average ± SD.

| Age (days) | Light Intensity (lux) | DW (µg) | Carbon (µg/ind) | Carbon (%) | Nitrogen (µg/ind) | Nitrogen (%) | C:N ratio |
|---|---|---|---|---|---|---|---|
| 0 | - | 87 ± 1 | 28.4 ± 0.5 | 32.5 ± 0.5 | 7.49 ± 0.10 | 8.57 ± 0.14 | 3.79 ± 0.06 |
| 2 | 0 | 106 ± 8$^{a}$ | 32.9 ± 3.8$^{a}$ | 31.0 ± 1.2 | 8.12 ± 0.91$^{a}$ | 7.64 ± 0.29 | 4.05 ± 0.07$^{a}$ |
| | 300 | 123 ± 6$^{b}$ | 40.2 ± 3.2$^{b}$ | 32.8 ± 1.2 | 9.58 ± 0.68$^{b}$ | 7.82 ± 0.20 | 4.20 ± 0.08$^{b}$ |
| | 1000 | 117 ± 6$^{ab}$ | 37.3 ± 3.2$^{ab}$ | 31.8 ± 1.4 | 8.85 ± 0.56$^{ab}$ | 7.56 ± 0.16 | 4.21 ± 0.10$^{b}$ |
| | 3000 | 124 ± 6$^{b}$ | 40.8 ± 3.1$^{b}$ | 32.8 ± 1.0 | 9.39 ± 0.65$^{ab}$ | 7.56 ± 0.19 | 4.34 ± 0.05$^{c}$ |

Different letters indicate significant difference ($p < 0.05$) among treatments.

**Table 6**. *Maja brachydactyla*. Influence of the light intensity (lux) on the dry mass and elemental body composition of newly moulted megalopae reared at 21 °C. Dry mass (DM), carbon content per individual (C), carbon percentage (C %), nitrogen content per individual (N), nitrogen percentage (N %) and C:N ratio. Values are expresed as average ± SD.

| Light Intensity (lux) | DW (µg) | Carbon (µg/ind) | Carbon (%) | Nitrogen (µg/ind) | Nitrogen (%) | C:N ratio |
|---|---|---|---|---|---|---|
| 0 | 193 ± 17 | 61.3 ± 4.6 | 31.9 ± 1.5$^{a}$ | 15.23 ± 0.89 | 7.93 ± 0.49$^{a}$ | 4.02 ± 0.08 |
| 300 | 181 ± 14 | 65.2 ± 5.9 | 35.9 ± 0.7$^{b}$ | 15.99 ± 0.80 | 8.83 ± 0.23$^{b}$ | 4.07 ± 0.17 |
| 1000 | 194 ± 22 | 65.6 ± 8.5 | 33.7 ± 1.3$^{a}$ | 15.95 ± 1.40 | 8.22 ± 0.28$^{c}$ | 4.10 ± 0.19 |
| 3000 | 199 ± 10 | 65.3 ± 4.4 | 32.7 ± 0.6$^{a}$ | 15.76 ± 0.51 | 7.91 ± 0.15$^{ac}$ | 4.14 ± 0.14 |

Different letters indicate significant difference ($p < 0.05$) among treatments.