Survival, development and growth of larvae of the blue swimmer crab, *Portunus pelagicus*, cultured under different photoperiod conditions

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Abstract

The blue swimmer crab is a commercially important species of the tropical Indo-Pacific regions that shows substantial potential as a candidate species for aquaculture. Optimization of larval rearing conditions, including photoperiod, is therefore important to establish a method for the intensive hatchery culture of this species. Newly hatched larvae of *P. pelagicus* in first zoeal stage (ZI) were reared under five photoperiod regimes 0L: 24D, 6L: 18D, 12L: 12D, 18L: 6D, and 24L: 0D (5 replicates per treatment) till they metamorphosed to megalopae (ranged from 8.5±0.3 days (18L: 6D) to 10.8±1.8 days (0L: 24D) at 29±1ºC). Daily, larvae of each treatment were fed an identical diet of mixed rotifer and *Artemia* nauplii, and the survival and molt to successive stages was monitored.

Newly hatched ZI larvae of *P. pelagicus* could successfully develop to the megalopal stage under all tested photoperiod conditions, but we detected significant differences in survival among treatments ($p<0.05$). The constant darkness treatment (0L: 24D) had the lowest (19.2±7.2%, mean±S.E.) cumulative survival from ZI to the megalopal stage, while the 18L: 6D treatment achieved the highest survival (51.2±23.6%). Similarly, the photoperiod significantly affected zoeal development. Constant darkness led to the longest cumulative zoeal duration (10.8±1.8 days), whereas the 18L: 6D treatment rendered the shortest larval development (8.5±0.3 days). In addition, larvae reared under constant darkness resulted in the smallest megalopae (carapace length=1.44±0.09 mm) and the lowest dry weight (0.536±0.188 mg).

In conclusion, photoperiod significantly affected the survival, development, and growth of *P. pelagicus* zoeal larvae. Constant darkness led to the lowest larval survival and developmental rate, while a photoperiod regime of 18L: 6D appeared to be the most suitable condition for the rearing of zoeal larvae of *P. pelagicus*.

Keywords: Blue swimmer crab; *Portunus pelagicus*; Larval culture; Photoperiod; Survival; Growth; Developmental duration.

1 INTRODUCTION

The blue swimmer crab, *Portunus pelagicus* (Crustacea: Decapoda: Brachyura: Portunidae), also known as sand crab, is widely distributed throughout the coastal and estuarine areas of the tropical Western Pacific and Eastern Indian oceans (Xiao and Kumar, 2004). With the growing popularity of this crab species, fishery harvests of sand crabs in these regions have increased since the early 1950's (Romano and Zeng, 2008). In 2003, landings reached 184,861 tonnes (source: FAO summary tables of fishery statistics; http://www.fao.org/fishery/statistics/en) and currently, blue swimmer crabs are largely sourced from fisheries that are unreliable and seasonal (Otto and Jamieson, 2003). Driven by the increasing demands, this has led to a growing interest in aquaculture of this crab. Furthermore, *P. pelagicus* has high aquaculture potential because it shows fast growth rates (Josileen and Menon, 2005), high fecundity, and relatively short larval duration (Romano and Zeng, 2008). As the aquaculture interest for this species increases, a better understanding of the basic larval culture conditions is necessary to optimize its production (Romano and Zeng, 2006).

The blue swimmer crab readily spawns in captivity and can spawn year round in the tropics. The larval cycle of *P. pelagicus* comprises four zoeal (ZI to ZIV) and one megalopal (M) stages (Shinkarenko, 1979; Arshad et al., 2006), and the total developmental time up to first crab stage is about two weeks (Romano and Zeng, 2008). Recent research on larval and juvenile rearing techniques have reported the use of formulated diets and various protein sources for *P. pelagicus* megalopae (Castine et al.,

2008), as well as salinity effects on survival, growth, and osmoregulation of early juveniles (Romano and Zeng, 2006). However, further research is required to identify culture conditions for other basic culture parameters, such as photoperiod.

To date, the literature offers no information on optimal photoperiod conditions for *P. pelagicus* larval culture, but generally, a photoperiod of 12 h light (12L) is adopted for its larval rearing (Castine et al., 2008). Unlike larval teleosts (Blaxter, 1980), larvae of decapod crustaceans are normally capable of feeding in the dark, suggesting that they do not have to rely on light or visual cues for forage (Gardner and Maguire, 1998). Still, light is an important abiotic factor that can substantially affect larval performance of crabs, including swimming, feeding behavior and growth (Sulkin, 1984; Minagawa and Murano, 1993; Minagawa, 1994; Gardner and Maguire, 1998). However, research on the effects of photoperiod on different aspects of decapod rearing have produced contradictory results among the different species and/or developmental stages studied so far (Anger, 2001). Although decapod crustacean larvae are considered non-obligated visual feeders, they may still utilize light for a more efficient feeding (Rabbani and Zeng, 2005). Indeed, evidence exists that newly hatched zoeal larvae of *P. pelagicus* fed three to four times more during daytime than at night time (Yatsuzuka, 1962, note that *Neptunus pelagicus* is a synonym to *P. pelagicus*).

In this context, we designed the present study to assess whether photoperiod affects the larval rearing success of *P. pelagicus.* The criteria used to measure this success were larval survival, growth and duration of the developmental stages. Our objectives were to contribute to optimize larval rearing techniques for *P. pelagicus* and to further examine the direct or indirect effects of light on the performance of decapod larvae.

2 MATERIALS AND METHODS

2.1 Broodstock crabs

Adult *P. pelagicus* were collected from the wild using baited traps in estuaries around Townsville (Queensland, Australia) and were transported to the Marine Research Facility Unit (MARFU) of the James Cook University (Townsville, Queensland, Australia) within a few hours of their collection. Males and unberried females were placed in a quarantine bath of seawater and formalin (100 µl L^{-1}) for 6 h and then transferred to 1000 L outdoor, re-circulating seawater tanks (temperature 28±2ºC, salinity 32±2‰). Every day the broodstock crabs were checked for spawning and fed to satiation on an alternation of prawns, mussels, and squid. Any berried female found was disinfected in a static formalin bath (50 µl L^{-1}) for 6 h before being individually transferred to a 300 L indoor tank (temperature 26±1ºC, salinity 34±1.5‰) for egg incubation and hatching. Water in the hatching tank was aerated and continually circulated through three cartridge filters (10, 5 and 1 µm) and a UV sterilizer. The berried females were not fed, and feces, discarded eggs, and other debris were siphoned out daily from the tank, accompanied with about a 10% water exchange.

2.2 Experimental design and setup

A single-factor experimental design was used to evaluate the effects of photoperiod on larval survival, development, and growth of *P. pelagicus*. Five photoperiod conditions were set up: 0L: 24D, 6L: 18D, 12L: 12D, 18L: 6D, and 24L: 0D (L=hours of light and D=hours of darkness), which were created by fluorescent light tubes and connected timers. The light intensity reaching experimental vessels was kept more or less constant at about 29 µmol m^{-2} s^{-1}, as measured with a LI-192SA Underwater Quantum sensor (Li-COR Biosciences, Lincoln, Nebraska, USA). The water temperature for all

photoperiod treatments was closely monitored (twice daily) and kept constant at 29±1ºC.

Newly hatched, vigorously swimming larvae (Zoea I, ZI) were collected from the hatching tank, counted, and randomly distributed into the experimental vessels (600 mL glass beakers filled with 500 mL UV-filtered seawater, 27±1‰ salinity, gently aerated) at 25 larvae per vessel. We set up five replicates for each photoperiod condition, hence a total of 25 experimental vessels were tested. Throughout the experiment, larvae were fed daily on a mixture of live prey composed of 40 rotifers mL^{-1} (ss-strain *Brachionus* sp.) and 5 *Artemia* sp. nauplii mL^{-1}. Rotifers were cultured with live microalgae (*Nanochloropsis* sp.) and harvested daily for the experiment, while *Artemia* nauplii were hatched daily from cysts (INVE Thailand, Amphoe Wachirabarami, Phichit, Thailand). Both rotifers and *Artemia* were fed directly to the larvae without enrichment. Every morning during the experiment, rotifers and *Artemia* were harvested from large-scale cultures. To determine the density of the harvested stocks, 3 x 1 mL samples were taken and counted to obtain the average using a Sedgewick-Rafter counter under a microscope. Based on this density, the volumes of rotifer and *Artemia* stocks required to achieve designated diet densities were calculated and added to new culture vessels (i.e. 600 mL beakers) that had been acclimatized at same water temperature of 29±1ºC. Afterwards, larvae in each replicate of a photoperiod treatment were counted, staged, and recorded. All surviving larvae were then transferred individually using a large bore pipette to a newly prepared culture vessel filled with fresh seawater and food. For the constant darkness treatment, daily checking and water exchange were conducted as quickly as possible, and in general, larvae exposure to light was less than 30 min per day, despite of this, for the simplicity of expression, 24L: 0D was used to describe the treatment throughout the text.

At the final stage of the experiment, any newly molted megalopae (M) found were immediately removed from the culture to avoid cannibalism. The carapace length (CL) of freshly molted megalopae was measured to the nearest 0.01 mm using a microscope (UNILUX-11, KYOWA, Tokyo). Dry weight (DW) was also measured to the nearest 0.001 mg on a CAHN C-33 microbalance after 24 h drying in a 50ºC oven. A replicate was terminated when all larvae within the replicate culture vessel had either molted to megalopal instar or died.

2.3 Statistical analysis

The rate (%) of cumulative survival to a particular larval stage was calculated as the number of larvae molted successfully to the next stage divided by the initial number of larvae in each replicate; data were square root transformed before the statistical analysis was performed. Larval development was expressed as the mean cumulative developmental duration to a particular larval stage, i.e., the average time required from the day of hatching to reach that particular larval stage. The coefficient of variation of the overall mean zoeal developmental duration (CV_{dv}) was calculated as the percentage of standard deviation of ZI to ZIV developmental duration divided by the mean developmental duration from ZI to ZIV. All measured parameters (survival rate, developmental duration, and megalopal CL and DW) were tested for normality. One-way ANOVA was performed to determine whether there were significant differences among different photoperiod treatments. Comparisons between groups after finding significant differences were performed by a Holm-Sidak test with an overall significance level of 0.05. All the statistical analysis was performed using a SigmaPlot 9 and SigmaStat 3 (Systat Software Inc., USA) software package.

3 RESULTS

3.1 Larval survival

Newly hatched zoeae of *P. pelagicus* developed successfully through their four zoeal stages under all photoperiod regimes tested, from constant darkness (0L: 24D) to constant light (24L: 0D) (Fig. 1). The overall zoeal survival to megalopal stage (i.e. cumulative survival at ZIV) increased concomitantly to the duration of the light phase up to 18 h per day (18L: 6D) and then decreased under constant light conditions (24: 0D). A significant difference ($p<0.05$) in the overall zoeal survival was detected between larvae kept under constant darkness (19.2±7.2%) and the 18L: 6D photoperiod (51.2±23.6%) (Fig. 1). However, no statistical differences in survival rates were found between the other treatments.

3.2 Larval developmental duration and synchronism

Table 1 shows the mean values of cumulative developmental duration for all zoeal stages under different photoperiod conditions. Statistically significant differences in larval development appeared as early as the first molt to the ZII stage, where constant darkness resulted in a significant delay in development (3.6±0.3 days) as compared to all other treatments ($p<0.05$). Under constant darkness, developmental duration to this stage was one day longer than the fastest development recorded under constant light (2.6±0.3 days). As larval ontogeny advanced, significant differences started to appear between other treatments too. We observed an overall trend of shorter developmental times as the duration of the light phase increased (Table 1). The shortest overall zoeal developmental duration was obtained under photoperiod 18L: 6D (8.5±0.3 days). Under this treatment conditions, we also observed the highest synchronism of molting to megalopal stage among individual larvae, as indicated by the lowest coefficient of variation of developmental duration (CV_{dv}= 3.3%) (Table 1). Although no significant

differences in the overall zoeal developmental duration were detected between the constant light and the 18L: 6D treatment, the CV_{dv} value under constant light nearly doubled that of 18L: 6D, indicating that substantially less synchronized molting occurred under constant light. The longest developmental duration was observed under constant darkness, where larvae took an average of 10.8±1.8 days to molt to megalopal stage. This duration is significantly longer than those found under the 18L: 6D and constant light treatments ($p<0.05$). Furthermore, larvae reared under constant darkness also had the highest CV_{dv} (17.0%) among all tested treatments, indicating the lowest synchronism at molting. No significant differences were detected between the developmental durations of the 6L: 18D and 12L: 12D photoperiod regimes (Table 1).

3.3 Dry weight and carapace length of newly settled megalopae

Figure 2 shows the mean dry weight and carapace length of newly molted megalopae from larvae reared under different photoperiods. Larvae reared under constant darkness resulted in the smallest megalopae (CL=1.44±0.09 mm) (Fig. 2b) and the lowest dry weight (0.536±0.188 mg) (Fig. 2a). Statistic analysis detected a significant difference in carapace length ($p=0.006$) of the newly settled megalopae between the constant darkness and the 6L: 18D treatment (CL=1.57±0.09 mm). Although larvae reared under the 6L: 18D treatment had the largest carapace length and the highest dry weight, the difference in dry weight between the two treatments was not statistically significant. Further, no significant differences in either carapace length or dry weight were detected among other treatments (Fig. 2).

4 DISCUSSION

In the present study, blue swimmer crab zoeae were able to develop through the whole larval cycle in the absence of light. However, the survival rate was significantly lower compared to that under photoperiod 18L: 6D. Similar results were observed in the

213 rearing of zoeae of the red frog crab *Ranina ranina*, where survival to megalopal stage
214 was significantly lower under either constant darkness or constant light conditions than
215 under a photoperiod of 12L: 12 D (Minagawa, 1994). However, some studies found that
216 photoperiod did not affect larval survival of the crabs *Pseudocarcinus gigas* (Gardner
217 and Maguire, 1998) and *Sesarma reticulatum* (Costlow and Bookhout, 1962) and the
218 spiny lobster *Jasus edwarsii* (Crear et al., 2003; Bermudes and Ritar, 2008). All these
219 observations indicate that the response to photoperiod might be species-specific;
220 therefore, we cannot establish any generalizations, and research needs to be conducted
221 on each species under study. Increasing the length of the light phase within a 24 h
222 photoperiod reduced the developmental duration of *P. pelagicus* zoeae. On the other
223 hand, a decrease in the day length caused significant delays and asynchronisms in their
224 successive molts. A similar response to day length has been commonly observed in
225 other decapoda crustaceans. For instance, phyllosoma larvae of *J. edwarsii* (stages I and
226 II) grew faster as a response to increasing day length (Bermudes and Ritar, 2008), and
227 larvae of *P. gigas* had shorter intermolt duration in treatments with longer photoperiods,
228 showing the most rapid development under continuous light (Gardner and Maguire,
229 1998). On the contrary, the accumulated zoeal duration of *R. ranina* was significantly
230 longer under 24L conditions than with the other treatments (Minagawa, 1994).
231 Our results confirm that zoeae of *P. pelagicus* do not rely only on visual cues for prey
232 capture or feeding since larvae could survive to megalopal stage under constant
233 darkness. However, larval survival, development, and growth under the 0L photoperiod
234 were significantly reduced, which suggests that light might promote feeding of the
235 larvae. This effect might occur either by stimulating zoeal swimming activity and,
236 hence, increasing the prey encounter probability or through other means that increase
237 the feeding efficiency of the larvae (Rabbani and Zeng, 2005). Thus, higher ingestion

238 rates under longer photoperiods resulted in shorter developmental duration and more
239 synchronic molts up to an optimum at 18L: 6D. Beyond this point, further increases in
240 day length reduced larval survival and increased developmental duration. This was
241 probably due to an energy imbalance caused by the high and lengthy swimming
242 activity, which was no longer compensated by increased food consumption. Also, the
243 larvae of *J. edwarsii* responded to light exposure with increased swimming activity,
244 feeding, oxygen consumption, and nitrogen excretion (Bermudes et al., 2008). In
245 contrast, light conditions did not influence either feeding rate or prey selection of larvae
246 of several decapod species, including the Atlantic mud crab *Panopeus herbstii* (Harvey
247 and Epifanio, 1997), the hermit crab *Pagurus bernhardus*, and the shore crab *Carcinus*
248 *maenas* (both in Dawirs, 1982).
249 The effect of photoperiod on *P. pelagicus* larval growth remains unclear. Dry weight of
250 newly molted megalopae was not significantly affected by photoperiod, even under the
251 0L: 24D treatment in which development was significantly retarded. Possibly, larvae
252 with the lowest nutrient reserves accounted for the substantially high mortality under
253 constant darkness, and only the strongest individuals were able to survive to megalopal
254 stage. Size (CL) was significantly lower in the 0L: 24D photoperiod than the 6L: 18D,
255 suggesting that under a relatively short light phase (6 h), larvae probably maintained a
256 relatively low daily metabolic rate and, hence, more energy could be used for growth.
257 Works with other species of crabs and lobster have also shown contradictory effects of
258 photoperiod regimes on larval growth (reviewed by Anger, 2001). For example,
259 Minagawa (1994) observed that *R. ranina* larvae grew more under continuous darkness
260 than under 6 to 24 h light conditions. In this case, larval metabolism could be lower
261 under constant darkness, and as a result, more energy could be channeled for growth.
262 Therefore, the larvae of *R. ranina* are probably more efficient feeders than *P. pelagicus*

263 under darkness. The high diversity of the response to light conditions -affecting larval
264 survival, development, and growth- of the various decapod crustaceans studied so far
265 suggests that their feeding mechanisms are likely very different (Rabbani and Zeng,
266 2005), which opens up a very interesting field for further research. Decapod crustaceans
267 show great diversity in larval stages and morphology, therefore it seems possible that
268 different feeding mechanisms have been adopted by various larval stages and species as
269 a response to day length changes.
270 In general, larval culture of *P. pelagicus* has been performed under a 12L: 12D
271 photoperiod. However, in the wild, *P. pelagicus* zoeae are found in large numbers in the
272 plankton of Australian subtropical and temperate waters in the late spring and summer
273 (Potter and De Lestang, 2000), when natural day length is longer than 12h. This
274 suggests that larval ingestion rates and energy balance might be well adapted to perform
275 under a photoperiod of more than 12 h, as we have confirmed in the present study. The
276 results obtained under continuous light demonstrated that *P. pelagicus* zoeae are well
277 adapted to develop under long photoperiods, but a relative reduction in survival rate
278 from 18L: 6D to 24L: 0D suggests that constant illumination produces suboptimal larval
279 culturing conditions. Based on our results, we recommend using an 18L: 6D
280 photoperiod in *P. pelagicus* zoeal rearing in order to improve larval development,
281 growth and survival.
282 Observations in *J. edwarsii* phyllosoma (Bermudes et al., 2008), where swimming
283 speed increased logarithmically with increasing light intensity, clearly indicate that not
284 only photoperiod but also light intensity can affect larval behavior. In the present study,
285 light intensity was kept constant for all photoperiod treatments and throughout the
286 experiment; therefore, further work is needed to determine if the results obtained here
287 are light intensity dependent.

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Table 1. Cumulative developmental duration (in days, mean ± S.D., *n*=5) during zoeal development of *Portunus pelagicus* reared under different photoperiod conditions. Different letters in superscripts within a same column denote significant differences (ANOVA; $p<0.05$). CV_{dv}= coefficient of variation of the overall mean zoeal developmental duration.

Photoperiod	**Cumulative developmental duration (days)**				
	ZI	**ZII**	**ZIII**	**ZIV**	**CV_{dv}**
0L: 24D	3.6 ± 0.3^{a}	5.6 ± 0.6^{a}	7.6 ± 1.0^{a}	10.8 ± 1.8^{a}	17.0%
6L: 18D	2.6 ± 0.1^{b}	4.7 ± 0.1^{bc}	6.8 ± 0.1^{ab}	9.4 ± 0.4^{ab}	4.1%
12L: 12D	3.0 ± 0.4^{b}	5.0 ± 0.4^{ab}	7.0 ± 0.4^{ab}	9.5 ± 0.5^{ab}	5.3%
18L: 6D	2.9 ± 0.4^{b}	4.3 ± 0.2^{c}	6.2 ± 0.3^{b}	8.5 ± 0.3^{b}	3.3%
24L: 0D	2.6 ± 0.3^{b}	4.5 ± 0.3^{bc}	6.3 ± 0.4^{b}	8.8 ± 0.6^{b}	6.5%

FIGURE CAPTIONS

Figure 1. Cumulative larval survival rate (%, mean ± S.E., *n*=5 replicates, 25 larvae per replicate) of *Portunus pelagicus* from hatching to megalopa reared under different photoperiod treatments. Different letters on the top of the bars denote significant differences between photoperiods within a stage (ANOVA, $p<0.05$).

Figure 2. Mean (± S.E., *n*=5) dry weight (DW, in mg) (a) and carapace length (CL, in mm) (b) of newly molted *Portunus pelagicus* megalopae from zoeal larvae reared under different photoperiod conditions. Different letters on the top of bars denote significant differences (ANOVA, $p<0.05$).

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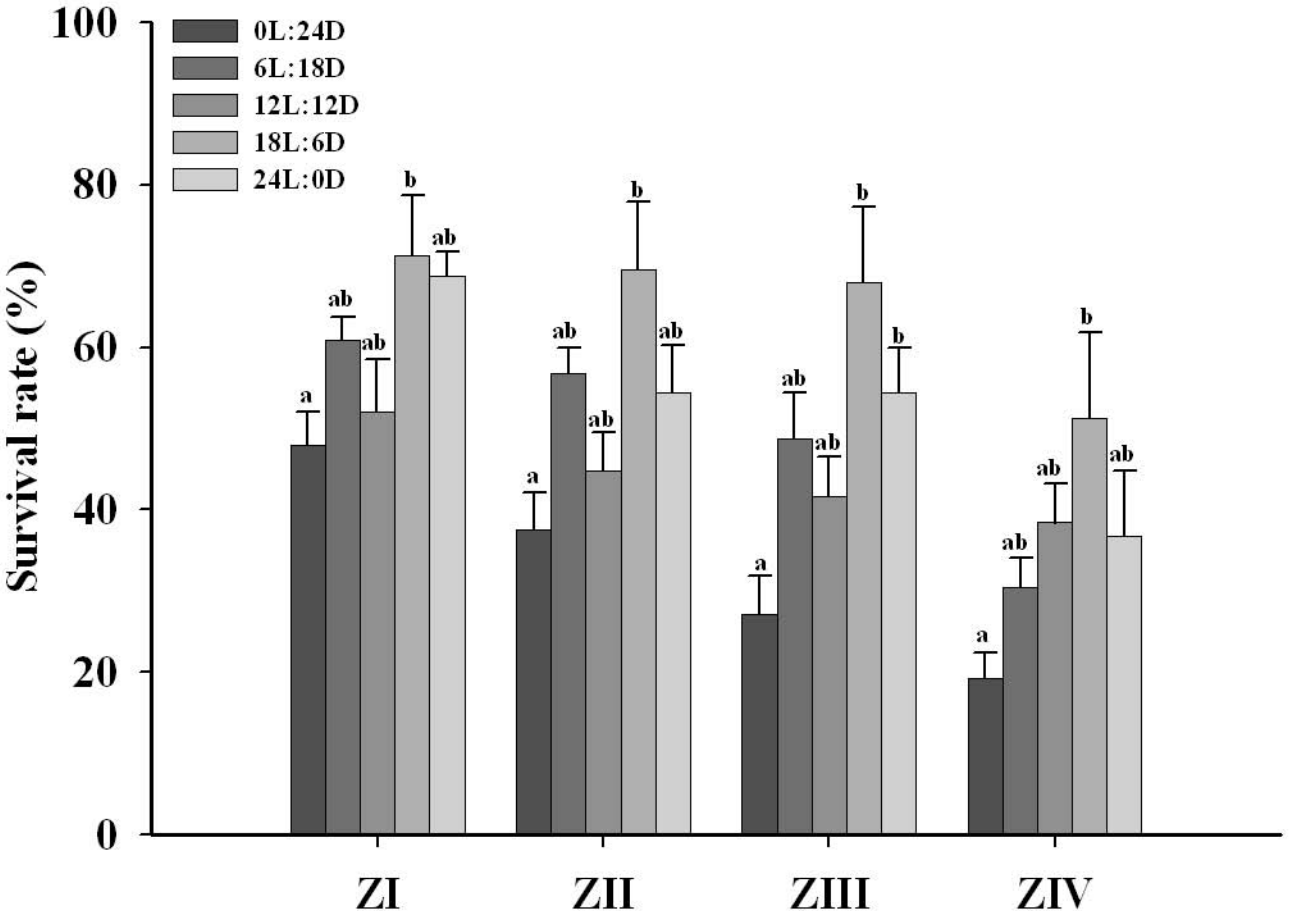
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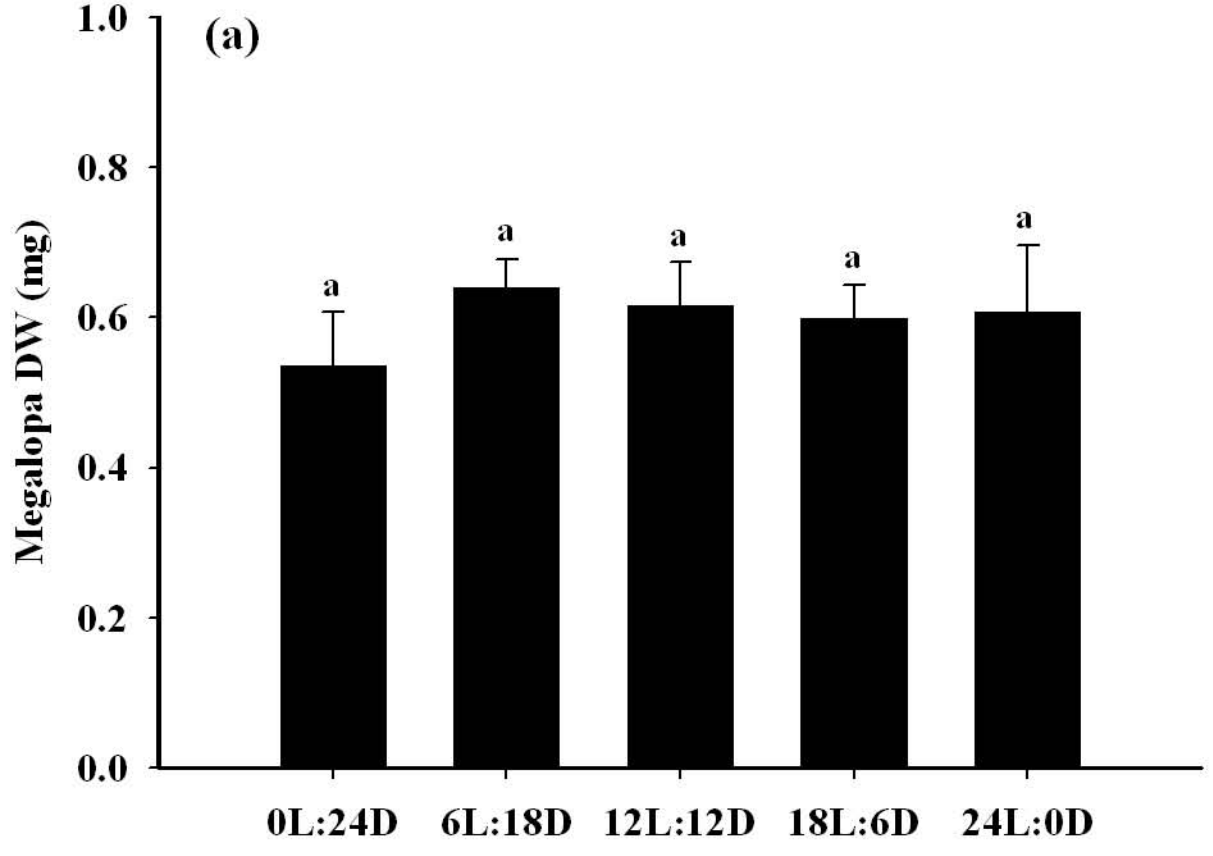
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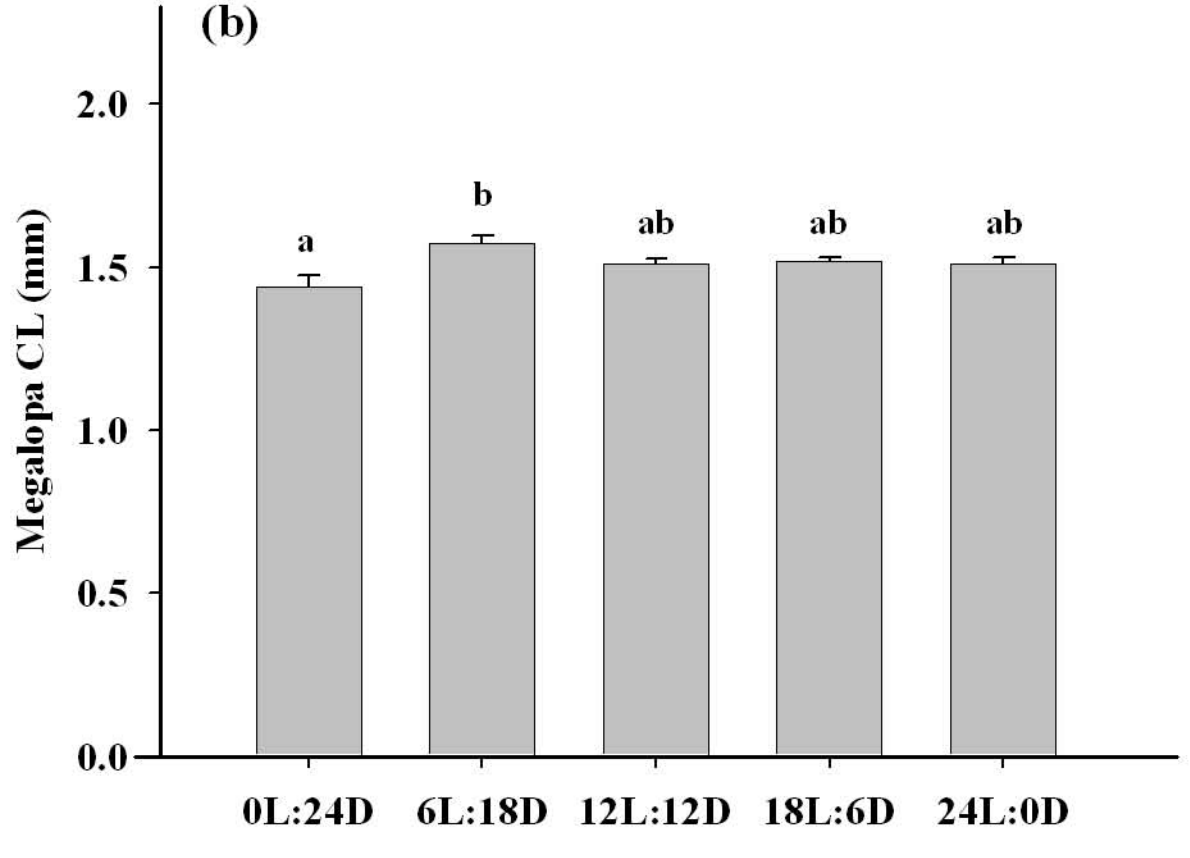
12L:12D
18L:6D
24L:0D


(b)
Megalopa CL (mm)
2.0
1.5
1.0
0.5
0.0
a
b
ab
ab
ab
0L:24D
6L:18D
12L:12D
18L:6D
24L:0D