Short communication

Effect of starvation during late megalopa stage of *Mithraculus forceps* (Brachyura: Majidae) on larval duration, synchronism of metamorphosis, survival to juvenile, and newly metamorphosed juvenile size

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Abstract

Larval resistance to temporary starvation is considered a key factor for successful development in the wild. Subjecting larvae to temporary starvation during early and/or late development is occasionally used in larviculture to reduce production costs. *Mithraculus forceps* is a popular species in the marine aquarium industry for their ability to control nuisance algae in aquarium tanks; a larval culture methodology was previously proposed in order to avoid collection from the wild. In an attempt to reduce production/feeding costs of *M. forceps* larval culture (two zoea stages and a megalopa), larvae (megalopa stage) were starved after 7 and 8 days post-hatch (DPH); starvation treatments were compared with those of the control treatment where larvae were fed continuously. No differences were found in survival to juvenile, metamorphosis synchronism or larval duration between the treatments, which suggest that at least by day 7 DPH, megalopae have already achieved the point-of-reserve-saturation (PRS) and are able to successfully metamorphose to crab stage without feeding (facultative lecithotrophic); newly metamorphosed juveniles are slightly smaller (particularly the ones starved after 7DPH). This flexible way of development might be very advantageous in the wild and might allow the reduction of costs of larval culture in captivity.

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1. Introduction

The ability of crustacean larvae to tolerate temporary starvation periods is believed to be essential for survival in the wild, particularly in unstable habitats, where food availability varies (Anger, 2001). Anger and Dawirs (1981) applied indices of larval starvation tolerance to crustacean larvae-the point-of-no-return (PNR), i.e. the average time of initial food deprivation that will not allow larvae to survive even after being fed again; and the point-of-reserve-saturation (PRS), i.e. the minimum time of initial feeding after which a food-independent development to the next stage is possible. These two indices have been used to compare the degree of nutritional vulnerability in different species and developmental stages among decapod species (Anger et al., 1981; McConaugha, 1985; Anger, 1987, 1995a, 1995b, 2001).

Subjecting larvae to temporary starvation is occasionally used in larviculture to reduce production costs (Simões et al., 2002), particularly during early development. This is used for larvae that hatch with adequate energy reserves allowing for the delayed onset of exogenous feeding (Mikami and Takashima, 1993; Schuh and Diesel, 1995a,b; Diesel and Schuh, 1998; Giménez and Anger, 2005). Studies on the effect of temporary starvation during later development are less common (Harms, 1992; Anger, 1995a,b; Harvey, 1996; McWilliam and Phillips, 1997); once larvae in the
last stage of development achieve the PRS, we can stop feeding to reduce cost. However, the suitability of these procedures should be studied in what matters not only to larval survival to juvenile and growth but also synchronism of metamorphosis and larval duration. It is important to have a good synchronism of metamorphosis to avoid larval size dispersion that could trigger competition and cannibalism (Polis, 1981; Smith and Reay, 1991; Hecht and Pienaar, 1993; Figueiredo and Narciso, 2006; Penha-Lopes et al., 2007). A long larval duration increases costs associated with manpower, electricity, diets, etc. Previous studies have shown that crustacean larvae subjected to temporary starvation may decrease survivorship, reduce growth rate and increase larval duration by performing mark-time moulting (Gore, 1985; Mikami et al., 1993; Schuh and Diesel, 1995a,b; Harvey, 1996; Diesel and Schuh, 1998; Calado et al., 2005; Giménez and Anger, 2005). These studies also showed that starvation impact on larval development is, in general, directly proportional to the time they are under those conditions (Mikami and Takashima, 1993; Giménez and Anger, 2005).

Marine ornamental crabs M. forceps (A. Milne Edwards, 1875) are popular in the aquarium industry for its ability to control nuisance algae. The direct effects of temperature, diet, stocking and prey densities on M. forceps larval and juvenile survival and growth were addressed by Penha-Lopes et al. (2005, 2006) who proposed a culture protocol to avoid collection from the wild. During the course of those experiments, G. Penha-Lopes noticed that the megalopae seem to feed less during the last two days prior to metamorphosis to newly metamorphosed juvenile (i.e. first crab stage); Anger and Dietrich (1984) reported some crustacean larvae reduce feeding rate in the pre-moult stage.

In an attempt to improve larval culture methodology of M. forceps and reduce production costs, we will examine if their megalopae require food 1 or 2 days prior to metamorphosis to crab. The suitability of this procedure will be evaluated based on survival to newly metamorphosed juvenile, metamorphosis synchronism, larval duration and newly metamorphosed juvenile size.

2. Materials and methods

2.1. Larvae culture

Experiments were carried out using a larval rearing system developed and described by Calado et al. (2003), and already used to culture M. forceps larvae by Rhyne et al. (2005) and Penha-Lopes et al. (2005). This system uses cylindrico-conical tanks and its main features are the “upwelling” flow that allows larvae and prey to be maintained in suspension and the use of screens that allow removing the prey from the tank without manipulation of the larvae. A photoperiod of 14L: 10D, salinity 35, and pH 8.0–8.2 were maintained for all experiments. M. forceps larvae development includes two zoeal stages (2 days per zoeal stage) followed by a megalopa (4 to 6 days).

In the control treatment, larvae were stocked in 10 l tanks at 10 larvae L−1 (N=100) and raised at 28 °C, and fed newly hatched Artemia nauplii at a density of 7 ml−1 (prey was renewed daily) until 15 day post-hatch (DPH) (according to the protocol proposed by Penha-Lopes et al. 2005, 2006). In the starvation treatments, larvae were raised under the same conditions except that they were starved after 7 or 8 DPH (2 and 1 day prior to first larvae undergoing metamorphosis, respectively). Four replicates were done for each treatment.

After the appearance of the first newly metamorphosed juvenile (crab), presence of crabs was checked daily, and crabs were removed from the system and recorded. At 9 DPH, a sample of 10 newly metamorphosed crabs from each replicate tank was used for measurements of carapace length (CL) and carapace width (CW) using an Olympus® SZ6045TR to the nearest 0.01 mm.

2.2. Statistical analysis

Models can be an advantageous approach when comparing to the traditionally used analysis of variance (Leung and Shang, 1989; Jorgensen and Bendoreichio, 2001; Figueiredo and Narciso, 2006; Penha-Lopes et al., 2007). Pinheiro and Bates (2000) developed the library “nlme” (non-linear mixed-effects models) for R 2.1.1. that allows predicting survival to juvenile through time and to statistically test final survival to juvenile, metamorphosis synchronism and larval duration (analysis of variance are incorporated on the development of the model). The percentage of larvae that survived to juvenile during the experiments (cumulative survivorship) was modelled using an asymptotic model (Eq. (1)) was used to describe the effect of late larval starvation on the survival to juvenile (y in %) through time (x in DPH):

\[
y(x) = \phi_1 \times \left(1 - e^{-e^{(x-x_0)/\phi_2}}\right), \quad y \leq 0.0
\]

In this formulation the model’s parameters (estimated using maximum likelihood) are:

- \( \phi_1 \) is the asymptote as \( x \to +\infty \) (Time \( \to +\infty \)) and represents the maximum survival to juvenile (%);
- \( \phi_2 \) is the logarithm of the rate of increase, corresponding to a half-life of \( t_{0.5} = \log^2(2)/\exp(\phi_2) \), which gives an idea on the synchrony of metamorphosis (greater values indicate greater synchrony);
- \( x_0 \) is the time (x) at which percent metamorphosis (y)=0, i.e., the day metamorphosis to crab began and gives an idea of larval duration.

The effect of the late megalopa starvation on maximum survival to juvenile (\( \phi_1 \)), synchronism of metamorphosis (\( \phi_2 \)), and larval duration (\( \phi_3 \)) were tested using analysis of variance (incorporated on the development of the model on “nlme”, described in detail by Pinheiro and Bates 2000). The development of
the model includes the performance of consecutive goodness-of-fit tests. A model was considered to be well fit when standardized residuals were inside 95% confidence level (between −1.96 and 1.96) and randomly distributed around zero.

CL and CW of newly metamorphosed juvenile (NMJ) from the different treatments were compared using one-way-ANOVA’s, followed by Tukey test if significant differences were found. Prior to ANOVA tests, data were checked for homogeneity of variances (Cochran’s test) and normality (Shapiro-Wilk’s test), and whenever necessary, data were appropriately transformed (Pestana and Velosa, 2006).

For all treatments, CL and CW of newly metamorphosed juveniles were modelled using Gaussian models since they can provide information on juvenile survival to newly metamorphosed juveniles (Fig. 1) was:

\[
F(x) = P(X < x) = \int_{-\infty}^{x} \frac{1}{\sqrt{2\pi}\sigma} \exp \left(-0.5 \times \left(\frac{x-\mu}{\sigma}\right)^2\right) \, dx
\]

and

\[
F(x) = P(X > x) = \int_{x}^{\infty} \frac{1}{\sqrt{2\pi}\sigma} \exp \left(-0.5 \times \left(\frac{x-\mu}{\sigma}\right)^2\right) \, dx.
\]

Parameters of the Gaussian model (μ and σ) were estimated by maximum likelihood (average and standard deviation of the sample, respectively) (Pestana and Velosa, 2006). Goodness of fit was tested by performing chi-square adjustment tests with Statistica 7.0.

Results were considered statistically significant when p-values were lower than 0.05 (Sokal and Rohlf, 1995).

3. Results

*Mithraculus forceps* larval development and survival (until 15 days post-hatch) for each treatment are represented in Fig. 1. The model that best described the effect of the tested starvation periods on percent survival to newly metamorphosed juveniles (Fig. 1) was:

\[
\text{Survival to NMJ} \% = (82.02) \times \left(1 - e^{-e^{[0.39 \times (DPH-8)]}} \right), \; y \geq 0.
\]

The development of this model with “nlme” provided the following results: late megalopa starvation did not significantly influence survival to juvenile (Φ1=0.8202%, p>0.83), synchronism on metamorphosis (Φ2=0.39, p>0.87) or larval duration (Φ3=8 DPH, p>0.79). The standard error of the estimated parameters is 3.84, 0.22 and 0.00, respectively for Φ1, Φ2, and Φ3.

Newly metamorphosed juvenile CW and CL were significantly different between the treatments (both p<0.001). Newlly metamorphosed juveniles that were fed throughout larval development had similar CL to those starved after 8 DPH (1.32±0.01 and 1.31±0.01 mm, respectively, p>0.60), and both were larger than those starved after 7 DPH (1.28±0.01 mm, both p<0.001). CW of the newly metamorphosed were significantly different between all the treatments (all p<0.01); individuals fed continuously measured 1.14±0.01 mm CW, while the ones starved after 8 DPH and 7 DPH measured 1.11±0.01 and 1.08±0.01 mm, respectively. These results are reflected in the Gaussian models developed (Fig. 2, whose parameters estimated by maximum likelihood and adjustment tests results are listed in Table 1).

4. Discussion

Traditionally only survival to juvenile (or even just percent survival) and growth are studied to optimize a larval culture
and cannibalism by larger larvae upon smaller ones (Polis, 1981; size dispersion, increase in mortality due to food competition maturation and reducing adult fecundity (Qian et al., 1990; by reducing juvenile growth and survival, increasing time to performance during grow out (Roberts and Lapworth, 2001) to juvenile (longer larval duration) can influence their Narciso, 2006). In some invertebrates, a delayed metamorphosis that need to be taken in account (Figueiredo and 1981; Gebauer et al., 2003). Metamorphosis asynchrony and size dispersion can also affect efficiency of juvenile culture. Coupled with the aggressive behaviour of larger crabs towards smaller conspecifics, different size crabs require different prey sizes and nutritional profile (e.g. Sainte-Marie and Lafrance, 2002).

The ideal conditions to raise M. forceps larvae would be those promoting high survival to juvenile, high synchronism of metamorphosis, and short larval duration, which means in an asymptotic model, a high \( \Phi_1 \), high \( \Phi_2 \) and low \( \Phi_3 \), respectively. The conditions that promote optimal growth are those that allow newly metamorphosed juveniles to achieve a large CL and CW with all individuals growing to a similar size, which means in a Gaussian model, a high \( \mu \) (newly metamorphosed juvenile CL and CW average) and a small \( \sigma \) (CL and CW standard deviation), respectively. A larger newly metamorphosed juvenile may reach adult size earlier (assuming that all juveniles grow at the same rate, independently of newly metamorphosed juvenile size) and therefore reduce grow-out period. A smaller range of newly metamorphosed juveniles CL and CW may imply that newly metamorphosed juveniles have similar conditions, thereby decreasing the risk of competition and cannibalism during juvenile culture (Polis, 1981; Smith and Reay, 1991; Hecht and Pienaar, 1993; Figueiredo et al., 2006; Penha-Lopes et al., 2007).

Modelling has been used to improve culture methodologies in captivity (Leung and Shang, 1989; Jorgensen and Bendoricchio, 2001). Models can be used as a decision support contributing to reduce production costs in aquaculture farms (Forsberg and Guttmersen, 2006). In aquaculture the main goal is to achieve high productivity at low cost. Since live prey culture is considered one of the most expensive and laborious activities of a hatchery, not providing Artemia nauplii to M. forceps larvae after 7 and 8 DPH (when considering 13 days of culture, 7 and 6 days of culture, respectively) could reduce M. forceps larval culture costs. Food is a key factor controlling survival, intermoult period and growth rate in crustacean larval culture (Mikami et al., 1993). In this study, M. forceps larvae subjected to starvation after 7 and 8 DPH (starting two and one day prior to metamorphosis to crab begins, respectively) displayed similar survival to juvenile, metamorphosis synchronism and larval duration to those continuously fed. Carapace width of newly metamorphosed juvenile subjected to starvation slightly decreases for every additional day of starvation but their size dispersion is similar (see Fig. 2) which means that food competition and cannibalism might not increase when megalopae are exposed to temporary starvation periods. Despite maintaining good survival, when starved after 7 DPH, larval growth is affected: average CL significantly reduces when compared with larvae starved after 8 DPH or continuously fed; and average CW is significantly smaller than the one displayed by continuously fed larvae. According to Knowlton (1974), larvae prioritize their energy use for (1) maintenance (survival), (2) moult and (3) growth. In optimal conditions larvae grow; in sub-optimal animals survive and moult (metamorphose) but do not grow. Subjecting megalopae to starvation after 7 and 8 DPH did not affect their survival and moulting period, but it also did not allow them to store enough energy that could then be used for growth (Fig. 2). From the biological perspective, the results suggest that if we fed M. forceps larvae with 7 Artemia nauplii ml\(^{-1}\), by day 7 post-hatch, megalopae have already achieved the point-of-reserve-saturation. In other words, megalopae at 7 DPH have gained sufficient organic matter or energy to successfully metamorphose to crab stage (Anger, 2001). Based on this experiment, we cannot determine if the PRS was achieved at 7 DPH or previously. Nutrition is known to primarily affect size increment at ecdisis, and has less importance on moulting frequency (Anger, 2001). As previous studies in other decapods have already showed, the greater the starvation period, the greater was the impact on larval development and growth (Mikami and Takashima, 1993; Mikami et al., 1993; Anger, 2001; Giménez and Anger, 2005).

From the aquaculture perspective, we believe starving M. forceps larvae after 7 DPH is already subjecting the larvae to sub-optimal conditions since growth is already significantly affected. This effect of starvation during larval development is thus carried over to juvenile stage since it affects their size and presumably their fitness (Giménez, 2004; Anger, 2006; Giménez, 2006). Considering these aspects, the best compromise between cost and profit might be starving them after 8 DPH (saving at least 5 days of Artemia nauplii feeding). A greater period of starvation produces a smaller newly metamorphosed juvenile that might require more time to achieve commercial size (1 cm CW); there may be reduced costs during larval culture but over-expenditure during juvenile grow out. However further long-term studies that could produce bio-economic data are needed to validate this suggestion.

Food availability in the plankton is considered a main factor accounting for fluctuations in abundance of planktrotrophic larvae (Thorson, 1950). Decapod larvae are able to sustain short periods of starvation if larvae previously acquire nutritional reserves to reach the PRS; larvae accumulate biomass to be used as energy source during subsequent development (Gore, 1985; Anger, 2001; Liddy et al., 2004). Larvae of many species of benthic marine invertebrates can delay settlement and metamorphosis in the absence of often highly specific external cues, such as prey items (Harvey, 1996). This study shows that at least by 7 DPH, M. forceps megalopae (fed with 7 Artemia nauplii ml\(^{-1}\)) are facultative lecitotrophic i.e., are tolerant to temporary starvation but retain the ability to capture and ingest prey when it becomes available. Lecithotrophy is generally considered an adaptation to
low or unpredictable food production in the environment where larval development takes place. It is usually more frequent in areas where nutritionally unpredictable conditions prevail such as high latitudes and freshwater environments (Anger, 1995a, 2001); however, even highly productive areas experience temporal or spatial variability in the plankton leading to a transitory or local occurrence of food limitation. A highly flexible mode of development can be determinant for survival, development and growth of larvae and other zooplankton in the wild (Thorson, 1950; Anger, 2001; Giménez and Anger, 2005); M. forceps megalopa ability to cope with short periods of lack of food might allow them to survive and metamorphose in areas with temporary food limitations.

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