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Productivity improvement of *Lysmata seticaudata* (Risso, 1816) larval rearing protocol through modelling

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Abstract

The Monaco shrimp *Lysmata seticaudata* (Risso, 1816) is a marine ornamental species whose ecology and biology, as well as its larval culture has previously been addressed. The objective of the study was to predict and improve productivity of this species rearing protocol through modelling. The models developed intend to help aquaculturists to maximize survival to postlarva, decrease larval duration and increase synchronism of metamorphosis and newly metamorphosed postlarvae size by manipulating temperature, diet, first feeding period and stocking density.

The models developed allow us to conclude that the *L. seticaudata* rearing protocol productivity can be improved by raising larvae at a density of 40 larvae L^{-1} and fed newly hatched *Artemia* nauplii since hatching to zoea V, and with Algamac 2000™ enriched *Artemia* metanauplii from zoea V to metamorphosis to postlarvae.

By providing more productive protocols to aquaculturists, destructive practices and wild collection may be reduced.

Keywords: *Lysmata seticaudata*; Ornamental shrimp; Larval culture; Modelling; Productivity

1. Introduction

The aquarium trade is a billion dollar business that may sustain continued growth for the next years (Bolker et al., 2002). In the past decade there has been a world-wide increase in the popularity of reef tanks, which has lead to an increased demand for marine ornamental species (Wood, 2001; Green, 2003). The vast majority of these organisms are still harvested from the wild, mainly in highly threatened coral reefs (Moe, 2003; Olivier, 2003). In order to decrease this pressure on the natural environment and, simultaneously, react to the growing demand of the hobbyists, inexpensive rearing techniques of these highly priced species should be determined (Tlusty, 2002; Corbin et al., 2003). Cultured animals are accustomed to captive conditions and more resilient than wild animals; additionally, hobbyists may be willing to pay extra for a higher quality product (Stime, 1999).

Presently, the percentage of commercially cultured ornamentals is limited to a few fish species, mainly clownfish of the genus *Amphiprion* (Wabnitz et al., 2003), some corals (Arvedlund et al., 2003) and marine decapods (Calado et al., 2003a). Tropical marine decapods are highly prized by traders and hobbyists, particularly from the genus *Hymenocera*, *Enoplometopus*, *Stenopus* and *Lysmata*. Their high market price, striking coloration,

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delicacy, and cleaning behaviour displayed by some species make them some of the most desirable marine ornamentals to be cultured (Moe, 2003). The development of a rearing system for ornamental decapod larvae by Calado et al. (2003b) has led to several breakthroughs in the culture of their larval stages, e.g., those in the genus *Lysmata* (Calado et al., 2005) and *Mithraculus* (Penha-Lopes et al., 2005). Utilizing a rearing system appropriate to decapod crustacean larviculture and manipulating biotic and abiotic factors, one can establish a larval culture protocol, capable of producing high quality animals at low cost as an alternative to wild harvested animals.

There are few ornamental species which development has already been investigated through all life cycle (Calado et al., 2003c), however, this knowledge has not been utilized along with models to predict or increase productivity. To avoid the collection of wild specimens, protocols and information on culture productivity should be addressed and be available for the aquaculture industry.

It is almost impossible to test one factor over its entire range. Instead, researchers select a few points within a range, and choose the value that causes higher survivorship and/or growth as optimum (e.g. Calado et al., 2005; Penha-Lopes et al., 2005). A comparison between treatments that detects if there are significant differences between treatments (such as analysis of variance) is not enough to select optimal conditions because the real optimum point may not be one of the few tested. To select the best conditions, extrapolation curves should be designed from available data, and optimal level for a factor estimated. However, optimal conditions do not necessary imply high productivity. Productivity models should be developed and provided to the aquaculturists, in order for them to select the most viable conditions to culture target species and predict productivity.

The Monaco shrimp *Lysmata seticaudata* (Risso, 1816) is a marine ornamental species from temperate and subtropical European waters (Calado et al., 2003c). This cleaner shrimp displays striking red coloration and is able to tolerate reef aquarium temperatures (Calado et al., 2003a). This species has also been utilized to control the pest-glass anemone *Aiptasia* (Calado and Narciso, 2005). The effect of several environment factors on the biology of this species, including the biological aspects of larval culture, has previously been addressed by Calado et al. (2005). However, through the use of models, one can predict and maximize production of captive raised animals. The postlarvae carapace length (CL) average and standard deviation obtained at the end of larviculture, as well as maximum survival to postlarva, larval duration and synchronism of metamorphosis can be predicted using models. By increasing productivity of aquaculture-raised individuals, we can protect natural environments, by decreasing the demand for wild harvested animals.

The goal of this study was to improve *L. seticaudata* rearing protocol productivity using models to predict and optimize survival to postlarva, newly metamorphosed postlarvae size from the production perspective by manipulating temperature, diet, starvation period and stocking density.

2. Materials and methods

These experiments were conducted in 2003 in Laboratório Marítimo da Guia (Portugal) and data used to construct the models were previously published by Calado et al. (2005). Larval culture experimental design and sampling methodology are briefly explained (for more details see Calado et al., 2005).

2.1. Larval culture experimental design

L. seticaudata larvae (from wild ovigerous females) were cultured in recirculating systems with several 10 L tanks (volume used by aquaculture companies to produce this shrimp in large scale) described in detail by Calado et al. (2003b). *L. seticaudata* larval rearing protocol was optimized sequentially: temperature, diet, starvation, stocking density and diet in higher stocking density (diet II). Experimental design is briefly described in Table 1, where the best result (in bold) from one experiment was carried over to all subsequent experiments. *Artemia* cysts (Unibest™ 020730) were hatched under standard conditions (Sorgellos et al., 1986). *Artemia* nauplii were enriched during 24 h with Algamac-2000™ (Aquafauna – Biomarine Inc.). Prey was provided daily at a density of 4 preys mL^{-1}. In all the experiments, four replicates (tanks) were used for each treatment.

2.2. Sampling methodology

For the treatment of total starvation (26 °C R10 S), all larvae were counted and their larval stage determined (according to Calado et al., 2004) every other day.

In all treatments, newly metamorphosed postlarvae (PL) were captured and counted daily for 15 days after the appearance of the first PL in each treatment. For each treatment, the carapace length (CL) of ninety randomly selected PL was measured under a stereo microscope (Olympus™, model SZ6045TR) with a micrometer eyepiece to the nearest 0.01 mm.

Table 1
Experimental design to test the effects of temperature, diet, starvation, stocking density and diet in high stocking density (Diet II) (N – newly hatched *Artemia* nauplii; M – 24 h-old unenriched *Artemia* metanauplii; EM – 24 h-old *Artemia* metanauplii enriched since hatch with Algamac-2000™; SZI+N – starved during zoea I stage and fed afterwards with newly hatched *Artemia* nauplii; NZV+EM – newly hatched *Artemia* nauplii to zoea V followed by 24 h-old *Artemia* metanauplii enriched with Algamac-2000™ from zoea V onwards; T – total starvation)

Experiment	Treatment	Temperature (°C)	Diet	Early starvation period (days)	Stocking density (larvae L^{-1})
1st: temperature	20R10N	20	N	0	10
	26R10N	**26**	N	0	10
2nd: diet	26R10N	26	**N**	0	10
	26R10M	26	M	0	10
	26R10EM	26	EM	0	10
3rd: Starvation	26R10S	26	–	T	10
	26R10SZI+N	26	SZI+N	2	10
	26R10N	26	N	**0**	10
4th: stocking density	26R10N	26	N	0	10
	26R20N	26	N	0	20
	26R40N	26	N	0	**40**
5th: diet II	26R40N	26	N	0	40
	26R40NZV+EM	26	**NZV+EM**	0	40

2.3. Statistical analysis and modelling procedure

The data on the effect of input variables (temperature, diet, starvation and stocking density) on survival to postlarva, newly metamorphosed PL size and productivity (only for stocking density) were modelled according to the diagram (Fig. 1).

2.3.1. Survival to postlarva model

The percentage of larvae that metamorphosed to PL throughout the experiments were modelled using library "nlme" (non-linear mixed-effects models) developed by Pinheiro and Bates (2000) in software R 2.1.1. We used a simple logistic model to describe the relationships between input and output data. According to Pinheiro and Bates (2000), the simple logistic (Eq. (1)) is a special case of the four-parameter logistic model where one of the parameters is set to zero.

$$y(x) = \frac{\phi_1}{1 + \exp[(\phi_2 - x)/\phi_3]} \quad (1)$$

In this formulation the model parameters (calculated using maximum likelihood) are

Φ_1 (since $\Phi_3 > 0$), is the horizontal asymptote as $x \to +\infty$ (Time $\to +\infty$) and represents the theoretical maximum survival to postlarva for that treatment.
Φ_2 is the x value at which the response is $\Phi_1/2$. It is the inflection point of the curve, which estimates time (in DPH) to 50% metamorphosis. It gives an idea of larval duration; the lower Φ_2, the shorter the larval duration.
Φ_3 is the distance on the x-axis between this inflection point and point where the y is $\Phi_1/(1+e^{-1}) \approx 0.73\Phi_1$. It gives an idea of the synchronism of metamorphosis; a lower Φ_3 means higher synchronism.

The effect of the factors (input variables) on maximum survival to postlarva (Φ_1), synchronism of metamorphosis (Φ_2), and larval duration (Φ_3) was tested using analysis of variance (incorporated on the development of the model on "nlme", described in detail by Pinheiro and Bates, 2000). All results were considered statistically significant at $p < 0.05$ level (Sokal and Rohlf, 1995). 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 outside 95% confidence level (between −1.96 and 1.96) were minimized and randomly distributed around zero.

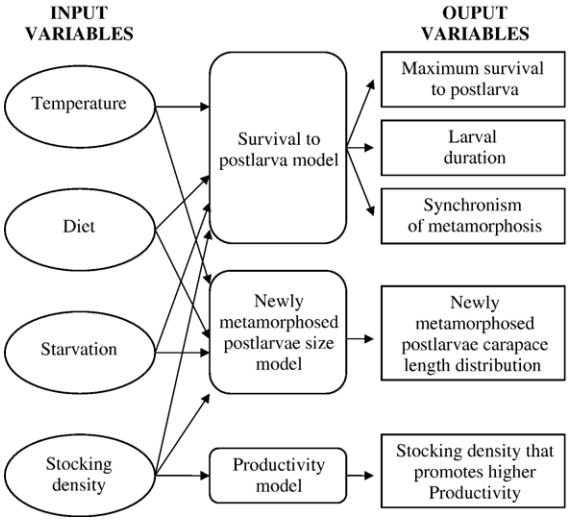

Fig. 1. Diagram representing input and output variables of the survival to postlarva, newly metamorphosed postlarvae size and productivity models.

2.3.2. Newly metamorphosed postlarvae size model

For all experiments, carapace length of newly metamorphosed postlarvae (PL) was modelled with Gaussian models since they can provide information on PL size obtained in the end of larval culture (and that will initiate juvenile culture) to producers.

Gaussian density function (Eq. (2)) graphically allows the determination of the most and least common CL that is obtained (Pestana and Velosa, 2002).

$$f(x) = \frac{1}{\sqrt{2 \times \pi} \times \sigma} \times \exp\left(-0.5 \times \left(\frac{(x-\mu)}{\sigma}\right)^2\right) \quad (2)$$

Gaussian distribution function (Eq. (3)) allows estimation of how many PL will be obtained until a certain size (multiplying $P[X<x]$ by the maximum number of PL that will be obtained), larger than a certain size (multiplying $P[X>x]$ by the maximum number of PL that will be obtained), and between y and x sizes (multiplying $[P(X<x)-P(X<y)]$ by the maximum number of PL that will be obtained) (Pestana and Velosa, 2002).

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

and

$$F(x) = P(X>x) = \int_{x}^{+\infty} \frac{1}{\sqrt{2 \times \pi} \times \sigma} \times \exp\left(-0.5 \times \left(\frac{(x-\mu)}{\sigma}\right)^2\right) dx \quad (3)$$

Parameters of the Gaussian model (μ and σ) were estimated by maximum likelihood (average and standard deviation of the sample, respectively) (Pestana and Velosa, 2002). The adjustment of the data to the model was done performing chi-square adjustment tests in software Statistica 7.0.

2.3.3. Productivity model

The polynomial model may be used to describe trend relationships between a measured response and independent variables. The linear, quadratic and cubic polynomial response curves provide good approximations to many relationships common to the biological and physical sciences. Since the levels of the factor stocking density are fixed and continuous, it makes sense to adjust a response curve that allows one to study the relationship of the response variable within untested values of an independent variable (Kuehl, 1994). In order to evaluate optimal stocking density, the productivity of a 10 L tank was calculated using as data the final number of postlarvae obtained in the end each stocking density treatment (multiplying percent survival by stocking density by tank volume for each replicate of the stocking density experiment). To build a response curve for optimizing productivity under a given stocking density, the orthogonal polynomials method was used (explained in detail by Kuehl, 1994). The polynomial model was fit to the observed data with computer regression models in Statistica 7.0. The estimated response curve is advantageous since it indicates the relationship between response and independent variable throughout the entire range of the independent variable used in the experiment (Kuehl, 1994).

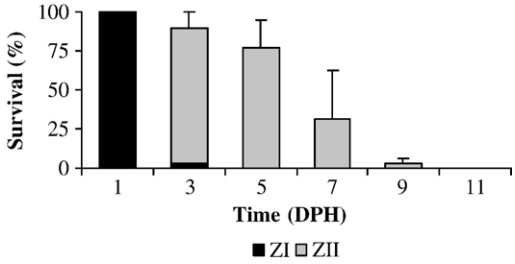

Fig. 2. Average (±standard deviation) survival (%) of larval stages (ZI - zoea I; ZII - zoea II) of *L. seticaudata* larvae cultured in absence of food (26° R10 S).

3. Results

Experimental data were presented in Calado et al. (2005) except treatment 26 °C R10 S. Larvae raised at 26 °C, stocked at 10 larvae L^{-1} and starved (26 °C R10 S) were able to moult to zoea II 3 DPH (days post hatch). Larvae remained in zoea II until total mortality was achieved 11 DPH (Fig. 2).

3.1. Effect of temperature

The model that best described the effect of the tested temperatures on percent survival to postlarva until a certain DPH (Fig. 3) was:

$$\text{Survival to postlarva (\%)} = \frac{94.16 - 10.64 \times A}{1 + \exp[(35.48 - 12.55 \times A - \text{DPH})/1.47]}$$

where $A=0$ for 20 °C and $A=1$ for 26 °C

The development of this model on "nlme" provided the following results:

- Maximum survival to postlarva was significantly higher ($p<0.043$) 10.64% at 20 °C ($\Phi_1=94.16\%$) than at 26 °C ($\Phi_1=83.52\%$);

- The day in which 50% of the viable larvae completed metamorphosis was significantly delayed ($p<0.001$) 12.55 days at 20 °C, in comparison with 26 °C ($\Phi_2=35.48$ DPH at 20 °C and $\Phi_2=22.93$ DPH at 26 °C);
- Synchronism of metamorphosis was not significantly different ($p>0.146$) at both temperatures ($\Phi_3=1.47$).

Gaussian models represented in Fig. 3 (whose parameters are listed in Table 2) show that postlarvae raised at 26 °C were larger, in average, than the ones raised at 20 °C, but displayed a wider range of carapace length (Table 2 and Fig. 3).

3.2. Effect of diet

The model that best described the effect of the tested diets on percent survival to postlarva until a certain DPH (Fig. 3) was:

$$\text{Survival to postlarva (\%)} = \frac{71.03-1.5\times A+11.83\times B}{1+\exp[(23.17-\text{DPH})/1.52]}$$

where $A=0$ and $B=0$ for 24 h-old *Artemia* metanauplii; $A=1$ and $B=0$ for 24 h-old *Artemia* metanauplii enriched with Algamac-2000™; $A=0$ and $B=1$ for newly hatched *Artemia* nauplii.

The development of this model on "nlme" provided the following results:

- Maximum survival to postlarva (PL) was significantly ($p<0.001$) influenced by diet. Larvae fed newly hatched *Artemia* nauplii displayed higher survivorship to PL ($\Phi_1=82.86\%$) than larvae fed unenriched and enriched metanauplii ($\Phi_1=71.03\%$ and 69.53% for unenriched and enriched metanauplii, respectively);
- The day in which 50% of the viable larvae completed metamorphosis was not significantly different ($p>0.934$) between treatments ($\Phi_2=23.17$ DPH);
- Synchronism of metamorphosis was not significantly different ($p>0.567$) between treatments ($\Phi_3=1.52$).

Gaussian models represented in Fig. 3 (whose parameters are listed in Table 2) show that postlarvae fed newly hatched *Artemia* nauplii had, in average, similar CL as the ones fed Algamac 2000™ enriched *Artemia* metanauplii, but displayed a wider range of carapace length. Postlarvae fed unenriched *Artemia* metanauplii displayed, in average, a smaller carapace length but a similar carapace length range than the ones fed Algamac 2000™ enriched *Artemia* metanauplii.

3.3. Effect of starvation

The model that best described the effect of the tested starvation period on percent survival to postlarva until a certain DPH (Fig. 3) was:

$$\text{Survival to postlarva (\%)} = \frac{86.68-21.13\times A}{1+\exp[(23.48-\text{DPH})/1.49]}$$

where $A=0$ for newly hatched *Artemia* nauplii (N) and $A=1$ for larvae starved them during zoea I stage (2 days) and then fed with newly hatched *Artemia* nauplii (SZI+N).

The development of this model on "nlme" provided the following results:

- Maximum survival to postlarva was significantly decreased ($p<0.001$) with starvation period ($\Phi_1=86.68\%$ and 65.55% for N and SZI+N, respectively);
- The day in which 50% of the viable larvae completed metamorphosis was not significantly different ($p>0.421$) between treatments ($\Phi_2=23.48$ DPH);
- Synchronism of metamorphosis was not significantly different ($p>0.053$) between treatments ($\Phi_3=1.49$);

Gaussian models represented in Fig. 3 (whose parameters are listed in Table 2) show that postlarvae starved during zoea I (2 days) were, in average, larger, but displayed a shorter CL range than the ones non-starved and fed newly hatched *Artemia* nauplii.

3.4. Effect of stocking density

The model that best described the effect of the tested stocking densities on percent survival to postlarva until a certain DPH (Fig. 3) was:

$$\text{Survival to postlarva (\%)} = \frac{83.59+4.52\times A-8.01\times B}{1+\exp[(22.95+0.17\times A+3.43\times B-\text{DPH})/(1.5-0.09\times A+0.77\times B)]}$$

where $A=0$ and $B=0$ for 10 larvae L^{-1}, $A=1$ and $B=0$ for 20 larvae L^{-1}, $A=0$ and $B=1$ for 40 larvae L^{-1}.

The development of this model on "nlme" provided the following results:

- Maximum survival to postlarva differed significantly ($p<0.033$) between treatments, decreasing 8.01% from 10 to 40 larvae L^{-1} ($\Phi_1=83.59\%$, 88.12% and 75.59% for 10, 20 and 40 larvae L^{-1}, respectively);
- The day in which 50% of the viable larvae completed metamorphosis was delayed significantly ($p<0.048$)

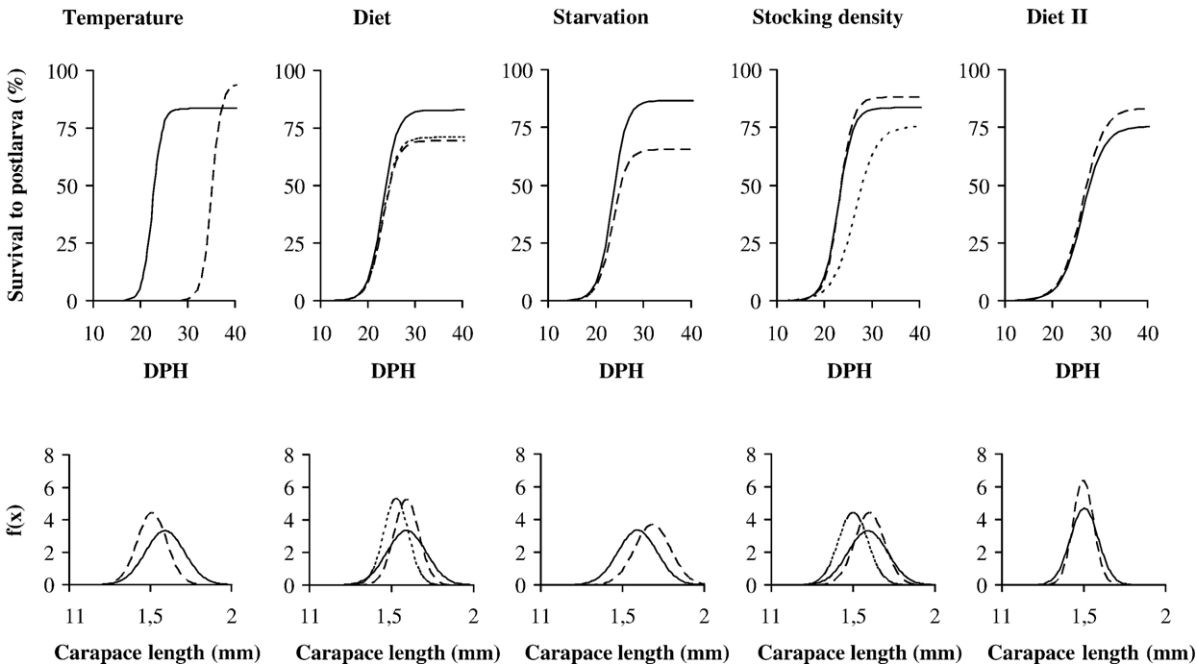

Fig. 3. Modelling percent cumulative survival to postlarva along time (DPH – Days Post Hatching) and carapace length (mm) of postlarvae that were reared under different conditions of: temperature −20 °C (20R10N in dashed line) and 26 °C (26R10N in solid line); diet – newly hatched *Artemia* nauplii (26R10N in solid line), 24 h-old unenriched *Artemia* metanauplii (26R10M in pointed line) and 24 h-old Algamac 2000™ enriched *Artemia* metanauplii (26R10EM in dashed line); starvation – newly hatched *Artemia* nauplii through ontogeny (26R10N in solid line) and starved during zoea I and fed afterwards with newly hatched *Artemia* nauplii (26R10SZI+N in dashed line); stocking density – 10 larvae L^{-1} (26R10N in solid line), 20 larvae L^{-1} (26R20N in dashed line) and 40 larvae L^{-1} (26R40N in pointed line); and diet in high rearing density (Diet II) – newly hatched nauplii (26R40N in solid line) and newly hatched *Artemia* nauplii and then switch to Algamac 2000™ enriched *Artemia* metanauplii after zoea V (26R40NZV+EM in dashed line).

with increasing stocking density, particularly at 40 larvae L^{-1} (Φ_2=22.95 DPH, 23.12 DPH and 26.38 DPH for 10, 20 and 40 larvae L^{-1}, respectively);

- Synchronism of metamorphosis was significantly different ($p<0.001$) between treatments, becoming less synchronous at 40 larvae L^{-1} (Φ_3=1.5, 1.41 and 2.27 for 10, 20 and 40 larvae L^{-1}, respectively).

According to the model, larvae stocked at 40 larvae L^{-1} are expected to take more 6 days to complete 75% metamorphosis (31 DPH), in relation to the ones stocked at 10 and 20 larvae L^{-1} (25 DPH).

Gaussian models represented in Fig. 3 (whose parameters are listed in Table 2) show that postlarvae stocked at 10 larvae L^{-1} had, in average, similar CL as the ones stocked at 20 larvae L^{-1}, but displayed a wider range of carapace length. Postlarvae stocked at 40 larvae L^{-1} displayed, in average, a smaller carapace length but a similar carapace length range than the ones stocked at 20 larvae L^{-1}.

Table 2
Parameters μ and σ of the Gaussian model that best adjusts to the carapace length (mm) of newly metamorphosed postlarvae for all the treatments (chi-square adjustment test results: Q-test statistic, *df* – degrees of freedom)

Treatment	Gaussian parameters		Adjustment test		
	μ	σ	Q	*df*	*p*-value
20R10N	1.51	0.09	13.93	9	0.12
26R10N	1.59	0.12	10.93	10	0.36
26R10EM	1.59	0.08	8.40	8	0.40
26R10M	1.53	0.08	14.45	8	0.07
26R10SZI+N	1.68	0.11	14.78	8	0.06
26R20N	1.60	0.09	17.66	16	0.34
26R40N	1.50	0.09	9.34	7	0.23
26R40NZV+EM	1.50	0.06	13.02	10	0.22

Stocking density strongly influences productivity ($p<0.001$): the quadratic equation was the best-fitting equation for the relationship between productivity and stocking density (SD in larvae L^{-1}) (Fig. 3):

$$\text{Productivity} = -30.89 + 12.58\ SD - 0.11\ SD^2 \quad (R^2 = 0.98),\ \text{for SD} \in [10, 40] \text{larvae L}^{-1}$$

Among the range of stocking densities tested, productivity increased with stocking density; 40 larvae L^{-1} was the stocking density that yields the highest productivity. Since the optimum of this model (56 larvae L^{-1}) was outside the range of values tested (10–40 larvae L^{-1}), it was impossible to validate such a prediction.

3.5. Effect of diet in high stocking density (Diet II)

The model that best described the effect of the tested diets on percent survival to postlarva until a certain DPH (Fig. 3) was:

$$\text{Survival to postlarva (\%)} = \frac{75.41 + 7.99 \times A}{1 + \exp[(26.29 - \text{DPH})/2.24]}$$

where $A=0$ for newly hatched *Artemia* nauplii and $A=1$ for larvae initially fed with newly hatched *Artemia* nauplii to zoea V followed by 24 h-old *Artemia* metanauplii enriched with Algamac-2000™ from zoea V onwards (26 °C R40 NZV+EM).

The development of this model on "nlme" provided the following results:

- Maximum survival to postlarva was significantly higher ($p<0.005$) 7.99% when enriched *Artemia* metanauplii was provided from zoea V onwards ($\Phi_1=83.4\%$) than feeding newly hatched *Artemia* nauplii during all larval stages ($\Phi_1=75.41\%$);
- The day in which 50% of the viable larvae completed metamorphosis was not significantly different ($p>0.883$) between treatments ($\Phi_2=26.29$ DPH);
- Synchronism of metamorphosis was not significantly different ($p>0.658$) between treatments ($\Phi_3=2.24$).

Gaussian models represented in Fig. 3 (whose parameters are listed in Table 2) show that postlarvae fed enriched metanauplii after zoea V displayed, in average, a similar CL as the ones fed newly hatched *Artemia* through ontogeny, but displayed a slightly narrower range of carapace length.

4. Discussion

The ideal conditions to raise Monaco shrimp larvae would be the ones promoting high survival to postlarva, short larval duration and high synchronism of metamorphosis, which means, in a logistic model, a high Φ_1, low Φ_2 and low Φ_3, respectively.

The conditions that promote optimal growth are those that allow postlarvae to achieve a large carapace length (CL) with all individuals growing to a similar size, which means, in a Gaussian model, a high μ (postlarvae CL average) and a small σ (CL standard deviation), respectively. A larger newly metamorphosed postlarva (PL) may arrive to adult size earlier (assuming all juveniles grow at the same rate, independently from each individual newly metamorphosed postlarva size) and, this way, reduce juvenile culture duration. A smaller range of PL CL may imply that PL have similar condition, thereby decreasing the risk of competition and cannibalism during juvenile culture.

4.1. Effect of temperature

According to the survival to postlarva model, while larvae reared at 26 °C displayed a 10.64% lower survival rate to PL than at 20 °C, their larval duration is considerably short at higher temperatures. From a commercial perspective it is more productive to raise larvae at 26 °C than at 20 °C. Since survival at 20 °C only increases 10.64%, the extra costs incurred for an additional 12–13 days culture period may not be justified. With consecutive cultures, tanks will be available to raise new batches much sooner at 26 °C. If newly hatched larvae are always available, considering larval duration and survival to postlarva, annual production would be much higher utilizing 26 °C: a single tank of 10 L, at 20 °C could produce 818 postlarvae ($365/42\times94.16\%\times100$), while a temperature of 26 °C would produce 1051 postlarvae ($365/29\times83.52\%\times100$). In both cases, if we consider more than one tank and/or a larger volume, the effect on productivity could be even greater.

As it was already shown by Calado et al. (2005), postlarvae reared at 26 °C displayed a larger CL than the ones reared at 20 °C, which might be due to the increase of both metabolic and ingestion rates at higher temperatures (Zhang et al., 1998). Larger postlarvae are preferred since they may achieve commercial size earlier.

4.2. Effect of diet

As was shown by Calado et al. (2005), the model of survival to postlarva developed in this study showed

unenriched or Algamac 2000™ enriched *Artemia* metanauplii did not improve survival to postlarva, metamorphosis synchronism, or larval duration. The need for research on other enrichment products might not be necessary since newly hatched *Artemia* nauplii yield a high postlarva survival rate (82.86%); enrichment products utilized through ontogeny would result in higher production costs and jeopardize the success of the commercial culture of these organisms.

Postlarvae fed unenriched metanauplii displayed a shorter CL average than the ones fed with recently hatched *Artemia* nauplii or Algamac 2000™ enriched *Artemia* metanauplii. According to Anger (2001), providing an inappropriate diet reduces growth between moults, resulting in smaller postlarvae. The Gaussian models showed that postlarvae that were fed with recently hatched *Artemia* nauplii displayed a similar CL average to the ones fed Algamac 2000™ enriched *Artemia* metanauplii, but display a higher standard deviation of CL. A small standard deviation of CL and, consequently, a small range of CL, is always preferable since it means individuals are more similar with each other, potentially reducing competition or cannibalism during the juvenile phase.

4.3. Effect of starvation

The zoea larvae of *L. seticaudata* hatch with enough yolk reserves to molt to zoea II, without exogenous feeding, indicating these larvae can be considered facultative lecithotrophs during the first larval stage (Anger, 2001).

The inception of first feeding is a determinant factor of crustacean larviculture survival and growth (Mikami et al., 1995). In order to minimize culture costs, starvation of the first zoeal stage is a common procedure, since newly hatched larvae generally have sufficient yolk reserves (Fletcher et al., 1995; Zhang et al., 1998); however, the consequences to larval survival and development rate were rarely investigated (Anger, 2001; Simões et al., 2002).

Although postlarvae that were starved during zoea I were larger than those non-starved and fed since hatch, maximum survival to postlarva decreases 21.13%. The profit associated with starving larvae during zoea I (2 days) might not compensate the lost in productivity, therefore, producers are recommended to feed *L. seticaudata* larvae right after hatch.

4.4. Effect of stocking density

Although low rearing densities generally result in higher survival rates (Simões et al., 2002), suitable higher rearing densities must be analysed in order to establish profitable culture methodologies (Goyert and Avault, 1978). According to the models developed, larvae stocked at 40 larvae L^{-1} displayed a lower maximum survival to postlarva (8.01% decrease), a longer larval duration and less synchronism in metamorphosis. Calado et al. (2005) recommended to culture *L. seticaudata* larvae at 20 larvae L^{-1} because this was the stocking density tested that promoted a higher survival rate. Although lower stocking densities are optimal for larvae survival, they are disadvantageous from a production perspective. Since percent survival decreases slowly with increasing stocking density, the number of postlarvae obtained in the end of larviculture actually increases with stocking density (until a certain point – optimum) which makes higher stocking densities usually much more productive. The model that best described the effect of stocking density on productivity showed that productivity increases with stocking density among the range of values tested (10–40 larvae L^{-1}). According to the model, the higher stocking density tested (40 larvae L^{-1}) is most productive. The model presented would be maximized at 56 larvae L^{-1}, however, this value is outside the range of values tested, so this prediction cannot be validated; future research should focus on higher stocking densities (Fig. 4).

Postlarvae reared at 40 larvae L^{-1} displayed a shorter CL which may be attributed to the low prey density (4 preys mL^{-1}). Consequently, treatments with higher stocking densities had a lower prey per larvae ratio (Knowlton, 1974; Welch and Epifanio, 1995).

4.5. Effect of diet on high stocking density (Diet II)

The ingestion of larger prey is energetically advantageous (Anger, 2001); however, the ingestion of *Artemia* metanauplii since hatch was revealed not to be so. According to several authors (McConaugha, 1985), prey size preference increases with larvae size. Thus, the best

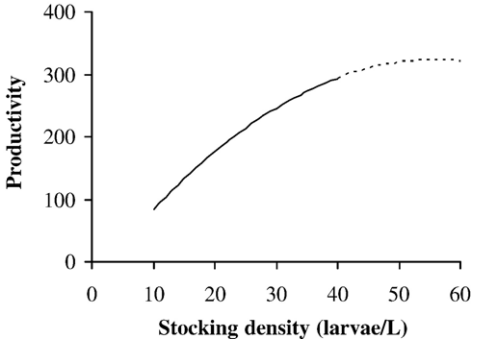

Fig. 4. Modelling productivity along a stock density gradient for a single 10 L tank (estimates from values tested – solid line; estimates from extrapolation – dashed line).

solution might be to initially provide smaller prey and at advanced larval stages provide larger prey (Figueiredo, 1975; Zhang et al., 1998; Calado et al., 2001).

Calado et al. (2005) considered that the use of the Algamac-2000™ did not produce significantly better results since survival at 15 days post metamorphosis (DPM) was similar to the one obtained with recently hatched *Artemia* nauplii, and therefore no need for enrichment. From the production perspective, the best evaluation criterion is not survival at 15 DPM (larvae and postlarvae altogether), but survival to postlarva. According to the models, the use of Algamac-2000™ enriched *Artemia* metanauplii from zoea V onwards increased survival to postlarva, and consequently production, 7.99%. With increased production, the cost associated with the use of this enrichment product may prove profitable, particularly when mass-culturing this species. The use of enrichment product had no effect on postlarvae carapace length; however, *L. seticaudata* larvae fed this diet developed to the postlarva stage with increased levels of docosahexaenoic acid (DHA) (Calado et al., 2005), indicating higher quality. Initiating juvenile culture with higher quality postlarvae might help achieving higher survival and growth rates, as well as resistance to diseases during the initial period of this stage.

5. Conclusion

The models presented (based on available data) allow us to conclude that, if newly hatched larvae are always available, the most productive conditions to raise *L. seticaudata* larvae is feeding them from the time of hatch with newly hatched *Artemia* nauplii to zoea V followed by Algamac 2000™ enriched metanauplii from zoea V onwards and stock 40 larvae L^{-1} (with 4 *Artemia* nauplii mL^{-1}). Due to the few temperatures tested with this species it is not possible yet to suggest an optimal rearing temperature. The temperature of 26 °C should be used until further temperatures are tested.

Possible interactions between different factors were not tested since available data were obtained from sequential experimental design. Factorial design experiments should be used in the future in order to test interactions between factors. Future studies should address higher stocking densities, higher prey density and tank volume and colour. A higher prey density could allow an increase in stocking density, and consequently, productivity. Other temperatures as well as diets (different live prey items, enrichment periods and products) should be tested and modelled in order to improve *L. seticaudata* larval rearing protocol.

It is strongly recommended to all producers to ally productivity predictions (such as the one provided in this paper) with economic predictors of costs (manpower, electricity, water, equipment, available space, broodstock, transportation cost and mortality, etc.) and profits (commercial size market prize) when deciding what is the most suitable protocol for their aquaculture facility in order to achieve the highest profitability.

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