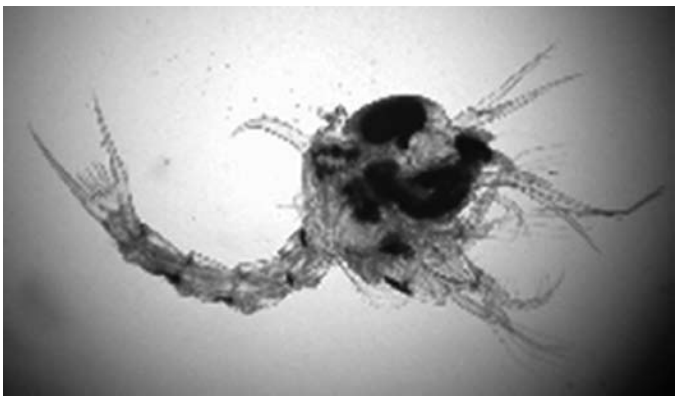
Productivity improvement of red clinging crab *Mithraculus forceps* through modeling

Joana Figueiredo[1]

A worldwide increase in the popularity of reef aquariums within the past decade has resulted in increased demand for marine ornamental species for the aquarium hobby trade. The aquarium trade is a billion dollar business that may sustain continued growth for years. The majority of organisms, 90 percent, are still harvested from the wild, particularly from highly threatened coral reefs in Southeast Asian countries where their collection represents a profitable income for natives. Destructive collection techniques include poisoning with cyanide and physical destruction of the reefs. Additionally, many animals die in transit before getting to retailers or home aquariums.

Fortunately, legislation to protect ornamental species and certifications to control the business has been increasing (Cato and Brown 2003). To reduce the pressure on the natural environment and satisfy the growing demand of hobbyists, inexpensive rearing techniques of highly prized and priced species needs to be developed. Captively raised animals are more resilient than wild animals as they are accustomed to aquarium conditions and often readily consume prepared food, such as pellets or flake food. For these reasons, hobbyists may be willing to pay extra for environmentally habituated animals. Presently, the percentage of commercially cultured ornamentals is still limited to a few fish species, mainly clownfish of the genus *Amphiprion*, some corals and a few marine decapods.

Organisms most desired by hobbyists for their home aquariums can be divided into four groups: the most beautiful and colorful such as clownfish, angelfish and surgeon fish; the strange and weird looking such as seahorses and eels; ones which blend in with the aquarium and challenge us to find them such as frogfish and stonefish and finally, the cleaners that assure your aquarium remains healthy, for instance. Organisms exhibiting cleaning behavior include pest controllers like the sea slug *Berghia verrucicornis*, peppermint shrimp *Lysmata wurdemanni* and Monaco shrimp *Lysmata seticaudata*, all of which control the pest glass-anemone *Aiptasia pallida*. The emerald crab *Mithraculus sculptus* and red clinging crab *Mithraculus forceps* control the pest bubble algae *Valonia* and *Ventricaria* (Figueiredo *et al.* in press). Other cleaners include macroalgae grazers, such as the scarlet reef hermit crab *Paguristes cadenati* and the scavengers and detritivores that include the bumble bee snail *Engina* sp. and *Nassarius* snail.



Mithraculus forceps *larvae (photographed by Gil Penha-Lopes)*

Aquaculture research tends to focus on the biological aspects of culture and often overlooks the production perspective when a culture protocol is developed. Some of the major goals required to aid the aquaculture industry are the optimization of protocols, particularly large scale culture, and production prediction. Modeling offers the advantage of using data from studies previously published to make predictions. As opposed to statistical analyses that will only reveal the relationships among the data, a model can offer a deeper understanding and simplified picture of reality and yield a better management plan. The biological aspects of culture are important to producers; however, this information needs to be adjusted for production. As an example, some of the questions for which a producer would like answers in larval culture include the following. What are the most productive abiotic and biotic conditions for larval rearing? How many juveniles will one obtain at the end of larval culture to initiate grow-out? Models, as opposed to statistical analysis, allow not only the comparison of survival to juvenile of the larvae produced, but also predict metamorphosis synchronism and day metamorphosis begins.

Rather than using final survival to evaluate how good a certain condition, for instance stocking density, productivity should be used. For example, for a 10 L tank, considering we have an unlimited supply of larvae, using a stocking density

Fig. 1. Crustacean larval rearing system where Mithraculus forceps larvae were raised to juvenile.

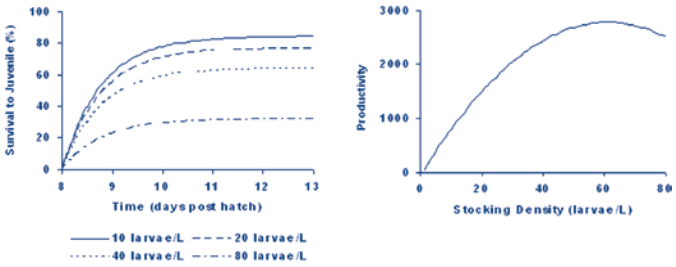


Fig. 2. Effect of stocking density (SD, larvae./L) on larval rearing.

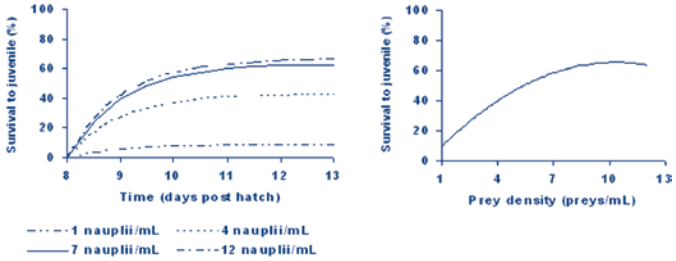


Fig. 3. Effect of prey density (PD, nauplii or prey/ml) on larval rearing.

of 10 larvae/L promotes 90 percent survival to the juvenile stage, while using a stocking density of 40 larvae/L promotes 60 percent survival to the juvenile stage. Based on survival, the lower stocking density appears to be a better choice but the lower stocking density only produces 90 juveniles, while the higher stocking density produces 240 juveniles. The prediction of this and other aspects are fundamental for good management of an aquaculture facility.

The knowledge of ornamental species culture has not been utilized in conjunction with models to predict and increase productivity. To reduce the collection of wild specimens, protocols and information on culture productivity should be addressed and be available to the aquaculture industry (Figueiredo and Narciso 2006). Through the use of models, one can predict and maximize production of captive raised animals. Survival to juvenile, larval duration and synchronism of metamorphosis are some of the various aspects that can be predicted using models.

Finding out the optimal conditions to raise an animal at each stage of development is not always straight forward because it is almost impossible to test one factor, such as temperature, over its entire range. Instead, researchers have been selecting a few values within a range and choosing as optimum the value that yields higher survivorship and/or growth. Following the example of temperature, a researcher cultures the desired species at four temperatures, within the range of temperatures the species occurs in nature, for instance 21, 24, 27 and 30ºC, compares the results achieved at each temperature, detecting if survival and growth significantly differed between treatments, through an analysis of variance, and finally suggests that producers use the tested temperature that promoted the highest survival and growth, for instance 27º C. An inherent problem arises because the optimum temperature may not be one of the tested values; who can guaranty the optimum temperature was not 28ºC? Instead, researchers should select the best conditions by extrapolating results that could be achieved within the temperature range of 21-30º C through the use of a response curve and use it to estimate the optimum temperature (see sidebars).

To exemplify how models can help improve productivity of aquaculture protocols we will use the larval and juvenile stages of the red clinging crab *Mithraculus forceps*. To construct the models, we will be using data obtained from the literature (Rhyne *et al.* 2005, Penha-Lopes *et al.* 2005, Penha-Lopes *et al.* 2006) where experiments were carried out using a larval rearing system developed and described by Calado *et al.* (2003). The system uses cylindrico-conical tanks and benefits from upwelling water flow that allows larvae and prey to remain in suspension and the use of screens that permit removal of prey items from the tank without manipulation of the larvae. Salinity of 35 g/L, pH of 8.0-8.2 and a photoperiod of 14L:10D were used in all experiments (Figure 1). Temperature, diet, stocking density and prey density tested during *Mithraculus forceps* larval and juvenile culture are presented on Table 1. Data obtained for survival and growth during larval and juvenile culture were used in the development of the models. Larval survival to juvenile was modeled with an asymptotic model. For stocking density, Survival to juvenile(%) = $\Phi_1 x(1 - e^{-e(0.25)x(DPH-8)})$ (Figure 2), where Φ_1=84.28% for 10 larvae.L^{-1}; Φ_1=76.71% for 20 larvae.L^{-1}; Φ_1=63.93% for 40 larvae.L^{-1}; Φ_1=31.83% for 80 larvae.L^{-1}. While for prey density, Survival to juvenile(%) = $\Phi_1 x(1-e^{-e(0.0041)x(DPH-8)})$ (Figure 3), where Φ_1=8.54% for 1 nauplii.mL^{-1}; Φ_1=42.61% for 4 nauplii/mL; Φ_1=62.96% for 7 nauplii/mL; Φ_1=66.09% for 12 nauplii/mL. Response curves were used to find the optimum stocking density and prey density during larval culture. Productivity was calculated by multiplying final survival to juvenile (%) by stocking density (SD) and tank volume (10 L) and the response curve is: productivity = -4.129 + 9.258 x SD – 0.076 x SD (Figure 2),

Table 1. *Mithraculus forceps* larval and juvenile culture conditions tested, protocol previously suggested, protocol suggested through modeling and productivity (per tank.year $^{-1}$ for larval culture and per m^2.year $^{-1}$for juvenile culture) improvement through modeling (NHA – newly hatched *Artemia*; EAM- 2days old *Artemia* enriched with Algamac 3050™; *Amphora* (microalgae); FNHA- frozen newly hatched *Artemia*; C.P. – commercial pellets; juv.- juveniles; * - it was not object of optimization modeling)

	Variable	Conditions tested	Protocol previously suggested	Protocol suggested after modeling	Productivity improvement
Larval Culture	Temperature	25ºC 28ºC	28ºC	28ºC*	-
	Diet	NHA EAM	NHA	NHA*	-
	Stocking density	10 larvae.L^{-1} 20 larvae.L^{-1} 40 larvae.L^{-1} 80 larvae.L^{-1}	40 larvae.L^{-1}	60 larvae.L^{-1}	50%
	Prey density	1 prey.mL^{-1} 4 preys.mL^{-1} 7 preys.mL^{-1} 12 preys.mL^{-1}	7 preys.mL^{-1}	10 preys.mL^{-1}	12%
	Temperature	25ºC 28ºC	28ºC	28ºC*	-
	Diet	NHA *Amphora* FNHA C.P. NHA + *Amphora* NHA + FNHA	NHA	NHA + *Amphora*	26%
Juvenile Culture		NHA + C.P.			
	Prey density	8 preys.mL^{-1}	8 preys.mL^{-1}	8* preys.mL^{-1}	-
	Stocking density	225 juv.m^{-2} 1130 juv.m^{-2} 3395 juv.m^{-2} 13580 juv.m^{-2}	3395 juv.m^{-2}	12900 juv.m^{-2}	280%

which optimum is 60 larva/L. For prey density, survival (%) = -3.14 + 13.24 x PD – 0.64 x PD^2 (Figure 3), which optimum is 10 preys/mL.

Diet effect on juvenile survival during growout was modeled with a logistic model

$$\textit{Juveniles' Survival} = 100 + \left(\frac{\phi_2 - 100}{1 + e^{\frac{(\phi_3 - x)}{4.42}}}\right)$$ (Figure 4),

where Φ_2=88.04 and Φ_3=6.19 for NHA, Φ_2=40.88 and Φ_3=15.54 for *Amphora*, Φ_2=68.42 and Φ_3=9.71 for FNHA, Φ_2=36.52 and Φ_3=8.41 for CP, Φ_2=60.85 and Φ_3=12.76 for NHA + *Amphora*, Φ_2=62.48 and Φ_3=8.92 for NHA + FNHA, and Φ_2=70.99 and Φ_3=8.92 for NHA + CP. The effect of stocking density was modeled with a linear (polynomial) model: Juvenile survival (%) = 100 + bx + cx^2 +dx^3

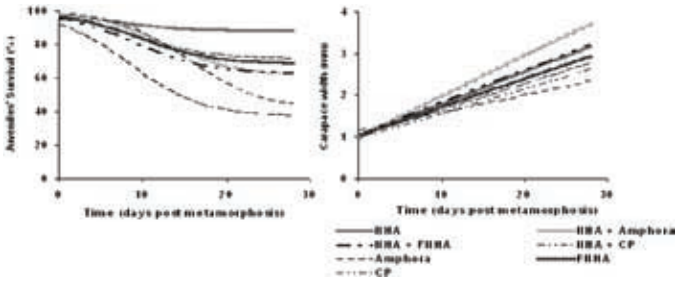


Fig. 4. Effect of diet and on juvenile culture.

(Figure 5), where x are days post metamorphosis and where b = - 3.6 , c = 0.17, and d = -2.65x10^{-3} for 226 crabs/m^2, b = -1.24, c = 0.02, and d = - 9x10^{-5} for 1132 crabs/m^2, b = -0.35

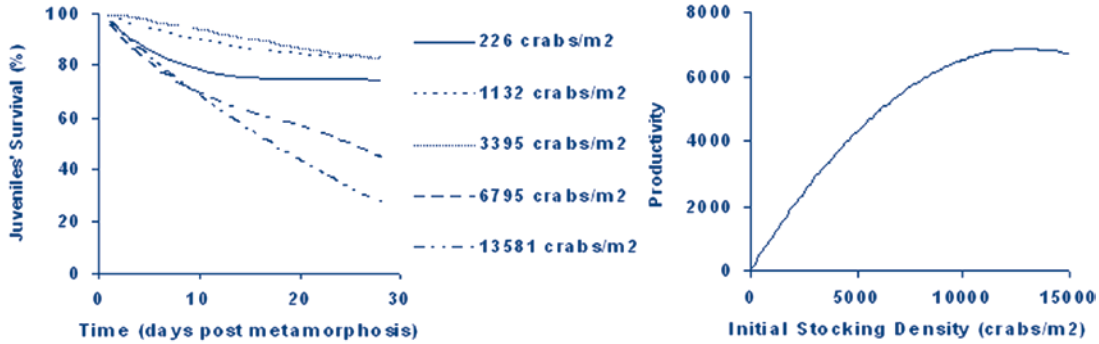


Fig. 5. Effect of stocking density on juvenile culture.

Mithraculus forceps *adults (photographed by Gil Penha-Lopes)*

, c = -0.03, and d = 7.5x10^{-4} for 3395 crabs/m^2, b = -4.69, c = 0.14, and d = -3.7x10^{-3} for 6791 crabs/m^2, b = -3.46, c = 0.03, and d = 4x10^{-5} for 13581 crabs/m^2.

Productivity (number of juveniles/m^2) was calculated by multiplying number survivors at 28 days post metamorphosis by stocking density. A response curve was used to find optimum stocking density during juvenile culture: productivity (%) = 1.065 x SD – 4.13x10^{-5} x SD^2 (Figure 5), which optimum is 12900 crabs/m^2.

The effect of diet on juvenile carapace width was modeled using linear models Juvenile CW (mm) = a + bx (Figures 4), where a= 1.0238 and b= 0.077 for NHA, a= 1.1462 and b= 0.0422 for *Amphora*, a= 1.0299 and b= 0.0686 for FNHA, a= 0.9505 and b= 0.0596 for CP, a= 1.0111 and b= 0.0971 for NHA + *Amphora,* a= 1.0361 and b= 0.0781 for NHA + FNHA, a= 0.9967 and b= 0.0633 for NHA + CP. The time to achieve commercial size (1 cm) was estimated based on these models (Figures 4): 117, 210, 131, 152, 93, 115, and 143 days for NHA, *Amphora*, FNHA, CP, NHA + *Amphora*, NHA + FNHA, and NHA + CP, respectively. Consult sidebars and Penha-Lopes *et al.* (2007) for further details.

The larval and juvenile survival and growth models developed suggest the use of a different protocol. By using a stocking density of 60 larvae/L and a prey density of 10 *Artemia* nauplii/mL during larval culture, and a diet that combines newly hatched *Artemia* and *Amphora* microalgae and 12,900 juveniles/m^2 during juvenile culture we were able to considerably improve the productivity of *Mithraculus forceps* culture (Table 1).

Ornamental aquaculture has been recognized as the best solution to minimize wild harvest from coral reef ecosystems, allowing a sustainable growth of the marine aquarium industry (Cato and Brown 2003). The models developed, besides being a very useful tool for production prediction, contributed to the improvement of productivity. Productivity models should be developed and provided to the producers to select the optimal conditions to culture target species (Figueiredo and Narciso 2006). By increasing productivity of aquacultured species, we can decrease the demand for wild harvested animals and protect natural environments. However, protocols still require adjustment for mass-scale culture and optimization combining bio-productive and economic predictors through modeling to maximize profitability.

Notes

[1]Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Estrada do Guincho, 2750-374 Cascais, Portugal. (Email: joana_figueiredo@portugalmail.pt.

Acknowledgment

Thanks to Justin Anto, Junda Lin, Luís Narciso and Gil Penha-Lopes for their help and constructive criticism. The author also thanks Fundação para a Ciência e a Tecnologia (FCT) and Portuguese Government for financial support (scholarship #SFRH/BD/17130/2004).

References

Calado, R., L. Narciso, S. Morais, A. L. Rhyne and J.Lin. 2003. A rearing system for the culture of ornamental decapods crustaceans larvae. Aquaculture 218: 329–339.

Cato, J. C. and C. L. Brown. 2003. Marine Ornamental Species: Collection, Culture and Conservation. Part III— The Invertebrates. Iowa State Press, United States.

Figueiredo, J. and L. Narciso. 2006. Productivity improvement of *Lysmata seticaudata* (Risso, 1816) larval rearing protocol through modelling. Aquaculture 261: 1249–1258.

Figueiredo, J., L. Narciso, R. Turingan and J. Lin. In press. Efficiency of using emerald crabs *Mithraculus sculptus* (Lamarck, 1818) to control bubble alga *Ventricaria ventricosa* (sin *Valonia*

ventricosa) (J. Agardh, 1887) in aquaria habitats. Journal of the Marine Biological Association.

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

Penha-Lopes, G., A. L. Rhyne, J. Lin and L. Narciso. 2006. Effects of temperature, stocking density and diet on the growth and survival of juvenile *Mithraculus forceps* (A. Milne Edwards, 1875) (Decapoda: Brachyura: Majidae). Aquaculture Research 37: 398-408.

Penha-Lopes, G., J. Figueiredo and L. Narciso. 2007. Modelling survival and growth of *Mithraculus forceps*' larvae and juveniles (A. Milne Edwards, 1875) (Decapoda: Brachyura: Majidae) in aquaculture. Aquaculture 264: 285–296.

Pinheiro, J. C. and D. M. Bates. 2000. Mixed-Effects Models in S and S-PLUS —Statistics and Computing. Springer-Verlag New York.

Rhyne, A. L., G., Penha-Lopes and J. Lin. 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.

Sidebar

Response curve

$y(x) = a + bx + cx^2 + dx^3$ (Equation 1)

Response curve is a linear (polynomial) model (Equation 1, where x is the variable, like stocking density or prey density) that allows us to predict survival or growth for the range of a variable, including values that were not tested, allowing us to find the optimum value for a certain variable. Response curves can only be applied to quantitative variables. To identify the polynomial effect: cubic, quadratic or linear, that adjusts better, we used the orthogonal polynomials method. These models were fit to the observed data with computer regression models in Statistica 7.0. The optimum value is estimated as the x value that produces the higher y (Figures 2, 3 and 4).

Linear and Non-Linear Models

The linear (Equation 4) and non-linear models, as in asymptotic and logistic models, (Equations 2 and 3), that allow us to predict results through time only for the tested levels of a variable (treatments), can be applied to both quantitative and qualitative variables and allow us to statistically test if parameters are significantly different between the different levels of the variable. Levels of a variable are the tested values of that variable, for instance 10, 20, 40 and 80 larvae/L are levels of the variable stocking density. The data are fit to the data using libraries "lme" and "nlme" developed by Pinheiro and Bates (2000) in software R. The program begins by estimating each parameter of the model (by maximum likelihood) for each one of the levels of the variable. The effect of the variable on each one of the parameters is tested using analysis of variance, incorporated in the development of the model. If a certain parameter is not significantly different between the different levels of the variable, the model will use the same value for that parameter for all levels of the variable, but if the parameter is significantly different between the different levels of the variable, the models will use a different value for the parameter for each level of the variable (Pinheiro and Bates 2000 for further details).

Asymptotic model

$$y(x) = \phi_1 \times (1 - e^{(-e^{\phi_2} \times (x - \phi_3))})$$ (Equation 2),

The asymptotic model (Equation 2, where x is time) has three parameters: Φ_1 is the asymptote as $x \to \infty$ and represents the final survival to juvenile (percent); Φ_2 is the logarithm of the rate constant, corresponding to a half-life of $t_{0.5} = \log 2/\exp(\Phi_2)$ which gives an idea on the synchrony of metamorphosis (greater values indicate greater synchrony); and Φ_3 is the value of x at which $y = 0$ indicating the time just before the first larvae is expected to metamorphose to juvenile or minimum larval duration (Figures 2 and 3).

Logistic model

$$y(x) = \phi_1 + \frac{\phi_2 - \phi_1}{\left(1 + e^{\frac{(\phi_3 - x)}{\phi_4}}\right)}$$ (Equation 3)

The logistic model (Equation 3, where x is Time) has four parameters: Φ_1 is the horizontal asymptote as $x \to \infty$ and represents the initial percent survival; Φ_2 is the horizontal asymptote as $x \to +\infty$ and represents the theoretical maximum survival for the treatment (level of the variable); Φ_3 is the x value at the curve inflection point which response is midway between the asymptotes and gives us an idea of the period of greater mortality during juvenile culture; Φ_4 is a scale parameter distance on the x-axis and gives us an idea of the mortality synchronism through development, a lower Φ_4 indicates higher mortality synchronism (Figure 4).

Linear (polynomial) Models

$y(x) = a + bx + cx^2 + dx^3$ (Equation 4)

The linear model (Equation 4, where x is Time) has four parameters: a, b, c and d. Unlike in the non-linear models, these parameters have no biological meaning (Figures 4 and 5).