Aquaculture Research, 2005, **36,** 278–283 doi: 10.1111/j.1365-2109.2005.01242.x

The effect of tank colouration on survival, metamorphosis rate, growth and time to metamorphosis freshwater prawn (*Macrobrachium rosenbergii*) rearing

David Yasharian, Shawn D Coyle, James H Tidwell & William Earl Stilwell

Kentucky State University, Aquaculture Research Centre, Frankfort, KY, USA

Correspondence: D Yasharian, Kentucky State University, Aquaculture Research Center, 103 Athletic Drive Frankfort, KY 40601, USA. E-mail: dyasharian@gwmail.ksu.edu

Abstract

Seedstock-costs of the freshwater prawn *Macrobrachium rosenbergii* can represent $>50\%$ of the total production costs. The purpose of this study was to evaluate the effect of culture tank colouration on survival, metamorphosis rate, weight and time required to reach the postlarval (PL) stage. Newly hatched prawn larvae were stocked into eighteen 16-L plastic culture tanks at a density of $30\,L^{-1}$. Tank colours evaluated were red, black, white, blue, green and yellow. Larval prawns were fed brine shrimp (*Artemia franciscana)* nauplii and a supplemental diet. Data indicated that larval tank colour had no significant impact ($P>0.05$) on the final PL size or days required to reach PL stage. However, total survival was significantly higher ($P<0.05$) in red and green tanks (84% and 78% respectively) than in white and blue tanks (56% and 44% respectively). Survival in the yellow and black tanks (71% in both) was not significantly different ($P>0.05$) from the red, green or white treatments, but was significantly higher ($P<0.05$) than the blue treatment. This could have a significant financial impact on commercial hatchery, and grow-out production, as most larval tanks currently in use are blue or black.

Keywords: freshwater prawn, larvae, tank colour

Introduction

Freshwater prawn (*Macrobrachium rosenbergii*) culture has become increasingly popular in temperate regions of the USA, and production is expected to increase (Woods, Murdock & Riggins 1998). Temperate culture includes three distinct phases of production: hatchery, nursery and pond growout. The hatchery phase is the most technically demanding and labour-intensive facet of the production cycle. As a result, seed-stock costs represent 40–50% of the total production costs in temperate climates (Woods *et al.* 1998; Dasgupta & Tidwell 2003). To facilitate continued growth of the fledgling prawn industry in the United States, the development of efficient hatchery production technologies, which improve survival and efficiency, will be required.

The impact of tank colouration on larval prawn growth and survival is a topic that remains controversial. Some prawn producers claim that a dark (black, blue, green) interior colour results in the highest survival (C. Upstom, pers. comm., 2003, Craig Upstrom, Aquaculture of Texas Inc. 4141 Fort Worth Highway Weatherford, TX 76087-8610). These producers suggest that larval prawn can detect their food more easily against a dark background, and larval distribution in the tank is more uniform thus ensuring proximity to food. However, others claim that larvae find their food mainly by a tactile response, rather than sight, and that white tanks make it easier to observe the larvae and the cleanliness of the tank (Rodrigues, Rodrigues & Moschen 1998). Lin and Omori (1993) demonstrated that feeding rate decreased with lighter tank colours, and that the best results were achieved using black tanks. However, Aquacop (1977) obtained their best production when larvae were produced in tanks with dark green walls. Daniels, D'Abramo and Parseval (1992) found that painting the lower 0.3 m of the tank walls and the bottom

of the tank beige in an 8-mt tank, while leaving the rest of the tank black, using an indirect light source, gave a good colour contrast to *Artemia*, allowing for more efficient feeding by larval prawns. Rodrigues and colleagues (1998) reported no evidence that tank colour affects the capture or ingestion of food; however, they observed an increased survival of postlarval (PL) in black tanks (55%) as opposed to white tanks (17%). Conversely Juarez, Holtschmit, Salmeron and Smith (1987) found that PL reared in black, blue and white tanks showed only 4% difference in survival (96%, 97% and 100% respectively).

The objectives of this study were to evaluate the effects of tank colouration on survival, metamorphosis rate, growth and time to metamorphosis of freshwater prawn larvae.

Materials and methods

Seven advanced berried females were placed into individual perforated containers and held in a communal 110-L hatch-tank. The hatch-tank was maintained at 29 °C using a 110-V bayonet heater (Visa-Therm Aquarium Systems, Loreggia, Italy). Larvae were allowed to hatch for 48 h in the hatch-tank, after which a random larval sample was obtained and preserved in ethyl alcohol. The culture containers were then stocked. Sampled larvae were later determined to be 99% stage I, and 1% stage II. Newly hatched larvae were removed from the hatch tank using a 100 μm dip net. The larvae were hand-counted into culture tanks using 100-mL pitchers. Larvae were stocked in rotations of 100, until the final density of 500 prawns per tank (30 L^{-1}) was obtained.

Eighteen 16.7-L plastic culture tanks were used, with three replicate tanks per colour treatment (red, black, white, blue, green and yellow), model NRC-90 (NAMPAC Raleigh, NC, USA). Tanks of the six treatment colours were randomized as to their location in the culture system. Prior to stocking, light intensity measurements (lx) were taken at the bottom of the tank (without water) and at the level of the water surface, approximately 10 cm below the top edge of the tank using a Sper Scientific broad range LUX/FC meter (Sper Scientific, Scottsdale, AZ, USA).

Each tank had a centre standpipe that was covered with 250 μm nylon screen for 10 days; after 10 days, the standpipe screen was changed to a 500 μm nylon screen. A settling basin received the effluent from all the tanks. This water was pumped to a common biofilter and then gravity fed back to the rearing tanks at a flow rate of 0.5 L min^{-1}. One week prior to stocking, biofilter media from an active recirculating system was introduced to the biofilter to inoculate the filter media with nitrifying bacteria.

Feeding rates were based on a feeding chart presented by D'Abramo, Daniels, Fondren and Brunson (1995). Based on this chart larval prawn were fed *Artemia nauplii*, *Artemia franciscana* (San Francisco Bay Brand, Newark, CA, USA) twice daily at 07:00 and 16:00 hours. *Artemia* were hatched in two 2-L *Artemia* cones and harvested using a 100 μm dip net. Harvested *Artemia* were then fed volumetrically using a 100-mL syringe. Beginning on day 6, *Artemia* feeding was supplemented by feeding a 'custard' comprised of egg, fish and squid as the main ingredients (Daniels *et al.* 1992). Larvae were fed the custard every day at 07:00 hours replacing the first feeding of *Artemia*. After day 6 *Artemia* were fed at 12:00 and 16:00 hours. Incoming water was turned off just prior to the feeding of *Artemia*, and remained off for 3 h after the *Artemia* had been introduced to prevent them from being flushed out of the tank before the prawn larvae consumed them.

A 1-hp regenerative blower (Metek Rotron Industrial Products, Saugerties, NY, USA) supplied air to each tank via 1.5-in air stones. Siphoning of solids was performed as needed. Total ammonia–nitrogen (TAN), nitrite–nitrogen, and pH were monitored every 3 days using a Hach Odyssey spectrophotometer (Hach Company, Loveland, CO, USA). Alkalinity was monitored every third day using a Hach digital titrator (Hach Company). Temperature, dissolved oxygen, and salinity were monitored daily using a YSI Model 85 oxygen meter (YSI Industries, Yellow Springs, OH, USA). Un-ionized ammonia was calculated based on TAN, temperature, and pH according to Boyd (1979). Water temperature was maintained between 28 and 31 °C by using an electric forced air wall heater to maintain ambient room temperature. Salinity was maintained between 12 and 14 g L^{-1} using a commercial sea salt mix (Crystal Seas brand salt mix, Baltimore, MD, USA). Larvae were exposed to a 12 L:12 D light cycle provided by two 48-in fluorescent full-spectrum bulbs suspended 18 in above each tank (Daniels *et al.* 1992).

Random samples of five larvae were collected from each tank on days 4, 7, 10, 13, 16, 19 and 22 of the study. Sampled prawn larvae were preserved in 100% ethyl alcohol, and examined under magnification to assess the developmental stage of each larva (Uno & Kwon 1969). An average larval stage index (LSI) value, which is a calculated weighted average

of stage determination (Manzi, Maddox & Sandifer 1977), was calculated for each tank for each sample date. Tanks were harvested when it was judged by visual assessment that all planktonic larvae had metamorphosed into benthic PL. All PL were hand-counted from each tank, and were weighed in bulk to determine total weight. Total weight was divided by total count to establish individual weight. Random samples of 50 PL from each tank were also weighed individually to the nearest milligram.

Statistical analyses

Effects of treatments on water quality and prawn growth and survival were evaluated by analysis of variance (ANOVA) (Zar 1989) using Statistix version 7.0 (Analytical Software, Tallahassee, FL, USA). If significant differences were indicated by ANOVA ($P \leqslant 0.05$), means were separated using a least significant difference (LSD) test (Zar 1989). Larval stage index values were compared using an ANOVA ($P \leqslant 0.05$). Means were compared using a LSD test to assess where the differences were found among treatments. The correlation between light intensity (lx) and survival between treatments was evaluated using a Pearson's correlation test (Zar 1989).

Results

Water quality

Over the duration of the study water temperature, salinity, and dissolved oxygen concentrations did not differ significantly ($P > 0.05$) between treatments. Average water temperature was 30.1 ± 0.1 °C, average salinity was $13.5 \pm 0.0\ g\ L^{-1}$, average dissolved oxygen concentration was $5.4 \pm 0.01\ mg\ L^{-1}$, and average alkalinity was $161\ mg\ L^{-1}$. Un-ionized ammonia (NH_3–N) concentrations averaged $0.110\ mg\ L^{-1}$ in the recirculating system over the duration of the study, with the highest concentration, $0.182\ mg\ L^{-1}$, occurring on day 21. Nitrite (NO_2–N) concentrations averaged $0.678\ mg\ L^{-1}$ for the duration of the study with the highest concentration, $1.164\ mg\ L^{-1}$ being recorded on day 21. The average pH for the duration of the study was 8.2. Water quality parameters were within recommended ranges for larval prawns (Armstrong, Stephenson & Knight 1976; Valenti, Mallasen & Silva 1998; Zimmermann 1998).

Survival and average weight

Harvest data indicated that larval tank colour had no significant impact ($P > 0.05$) on the final PL weight, which averaged 9 mg. Total survival (Table 1) was significantly higher ($P < 0.05$) in the red and green treatments (84% and 78% respectively) than the white and blue treatments (56% and 44% respectively). Survival in the yellow and black treatments (71% and 71%) was significantly higher ($P < 0.05$) than the blue treatment (44%), but not different from the red, green and white treatment (84%, 78% and 56% respectively).

Larval development and time to metamorphosis

Larval development (Table 2) was not significantly different ($P > 0.05$) on sample days 4, 7, 10, 16, 19 or 22. However, on day 13, the larvae sampled in the white treatment had a significantly lower ($P < 0.05$) LSI value than the other treatments. There was no significant difference ($P > 0.05$) in the days required to reach the final PL stage (Table 1).

Light intensity

All tanks, although exposed to identical light sources, showed significant differences ($P < 0.05$) in light intensities (lx) at the water surface, as well as the bottom of the tank (without water). The highest lux value at the tank bottom was in the white culture

Table 1 Survival and average weight (mean ± SD) and time to reach metamorphosis of *Macrobrachium rosenbergii* larvae grown in different colour tanks

Treatment	Survival	Average weight (mg)	Time to metamorphosis (days)
Red	83.9 ± 3.6^{a}	8.9 ± 0.3	25
Green	77.6 ± 16.2^{a}	8.6 ± 0.4	25
Yellow	71.2 ± 2.6^{ab}	9.1 ± 0.1	25
Black	71.0 ± 7.5^{ab}	9.6 ± 0.4	25
White	56.4 ± 5.7^{bc}	8.4 ± 0.3	25
Blue	44.4 ± 0.7^{c}	9.5 ± 0.8	25
	$P = 0.005$	$P = 0.165$	

Means with identical superscripts in common columns are not significantly different ($P < 0.05$).
Means without superscripts in common column have no significant difference ($P > 0.05$).

Table 2 Larval stage index (LSI) (mean ± SE) for *Macrobrachium rosenbergii* larvae grown in different colour tanks

Tank colour	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22
White	3.3 ± 0.18[a]	4.5 ± 0.53[a]	6.5 ± 0.53[a]	7.1 ± 0.07[b*]	8.5 ± 0.64[a]	8.5 ± 0.64[a]	11.2 ± 0.23[a]
Blue	3.7 ± 0.13[a]	5.0 ± 0.24[a]	6.6 ± 0.40[a]	7.7 ± 0.18[a]	8.3 ± 0.13[a]	8.3 ± 0.13[a]	9.6 ± 1.02[a]
Black	3.4 ± 0.12[a]	5.2 ± 0.20[a]	7.0 ± 0.00[a]	7.7 ± 0.27[a]	9.3 ± 0.24[a]	9.3 ± 0.24[a]	11.2 ± 0.20[a]
Red	3.7 ± 0.13[a]	5.7 ± 0.24[a]	6.9 ± 0.07[a]	7.9 ± 0.07[a]	9.1 ± 0.24[a]	9.1 ± 0.24[a]	11.1 ± 0.37[a]
Green	3.7 ± 0.18[a]	5.5 ± 0.13[a]	7.1 ± 0.13[a]	8.3 ± 0.07[a]	9.2 ± 0.20[a]	9.2 ± 0.20[a]	11.3 ± 0.13[a]
Yellow	3.7 ± 0.12[a]	5.7 ± 0.07[a]	6.9 ± 0.07[a]	8.1 ± 0.29[a]	9.2 ± 0.12[a]	9.2 ± 0.11[a]	11.5 ± 0.13[a]

Means with identical superscripts in common columns are not significantly different.
*Significant difference ($P < 0.05$).

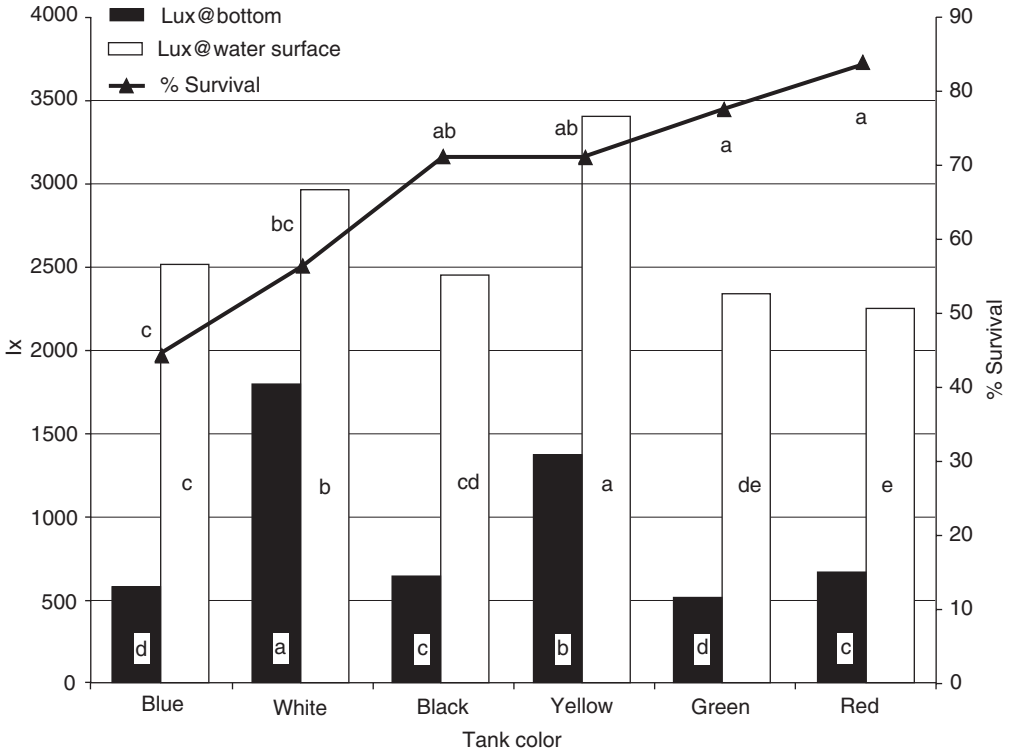

Figure 1 Correlation between light intensity (y_1-axis) at the bottom of an empty tank and at the water surface, and prawn survival (y_2-axis). Different letters between treatments within measured parameters indicate significant differences ($P < 0.05$). Pearson's correlation analysis indicated no significant correlation ($P > 0.05$) in between light intensity (lx) and prawn survival.

tanks with average value of 1804.5 ± 23.3 lx, and the lowest value at the bottom of the tank was the in the green culture tanks with an average value of 519.0 ± 19.0 lx. The yellow culture tanks had the highest lux value at the surface of the water with average value of 3400.0 ± 155.24 lx, the lowest value at the water surface was found in the red culture tanks with an average value of 2250.0 ± 26.5 lx. However, there existed no correlation ($P > 0.05$) between light intensity and survival (Fig. 1).

Discussion

Survival in prawn larvae is assumed to be directly related to maintenance of good water quality, food quality and quantity, and the ability of the prawns to acquire the food (Armstrong *et al.* 1976; Aquacop 1983). Since all treatments in this study shared the same water quality and feeding regimen, survival differences must be related to the ability of the larvae to acquire food; the most significant variable tested in

this study was tank colouration, suggesting that this was the factor concerned. Lin and Omori (1993) found that feeding rates of larval *M. rosenbergii* decreased with the increase in lightness of the rearing container. Survival in the green culture tanks were not statistically lower than the red culture tanks which showed the highest survival; this agrees with the findings of Aquacop (1977) who obtained their best survival in larval tanks with dark green walls. It has been suggested that the early larval stages of *M. rosenbergii* are non-active hunters and that although they have a good visual power (Daniels *et al.* 1992) they seem to capture food by random encounter (Moller 1978). As larval development progresses, visual ability and active predatory behaviour increases. As larval prawn become active predators, the ability to see the food item becomes increasingly critical. The ability to contrast or silhouette food items against a background is important in an aquatic environment. Ostrowski (1989) found that dolphin (*Coryphaena hippurus*) larvae grown in tan coloured tanks fed a diet of rotifers – also tan coloured – tended to continuously shake their heads from side to side, apparently in an attempt to focus on the food item. Larvae grown in black tanks did not exhibit this behaviour. If a food item does not visually stand out well against a background, it will be difficult to capture and consume resulting in a poor success rate and higher mortalities for the prawn PL.

It may well be that there are certain critical phases of larval development when tank colour is most important, such as the period or transition from random encounter feeders to visual predators. After this transition period, background colour may play less of a role. If this theory holds true, larval prawns in certain coloured tanks (white and blue) could not detect sufficient food for a short period resulting in the starvation or cannibalization of smaller weaker animals. After this critical period, those prawns that survived would grow and mature at a normal rate. This may explain why time to final metamorphosis and average weights were not significantly different among treatments, although survivals were.

Another theory is that some background colours may represent chronic stressors for larval prawn. Lin and Omori (1993) found that swimming behaviour of *M. rosenbergii* larvae differed significantly with the colouration of culture containers. They found that the speed and distance of horizontal movement of the larvae increased by three times in white containers, as compared with those grown in the control containers (black). They concluded that excess 'excitation' in lighter coloured containers may be responsible for decreased feeding rates. This would also suggest an increased consumption of energy, resulting in a smaller 'scope for growth'. In some fish, the presence of certain colours or light intensities can cause a chronic stress reaction. Fanta (1995) found that Nile tilapia (*Oreochromis niloticus*) grown in white tanks showed increased respiratory rates, swimming speed, and aggressive behaviour compared with fish grown in green tanks. Papoutsoglou, Mylonakis, Miliou, Karakatsouli and Chadio (2000) found that carp (*Cyprinus carpio*) grown in white tanks exhibited less stress, better growth and a lower plasma cortisol level, an accepted indicator of fish stress (Barton & Iwama 1991), than carp grown in green or black tanks. Tank colouration may serve as a calming agent allowing the prawn to feed more readily, as well as reducing cannibalistic behaviour. Tank colours that most mimic the natural environment (green, tan, brown, grey and orange) in which the species evolved may be more productive than tank colours that are rarely found in a natural environment (blue, white, violet, yellow, pink and silver). This appears to be the trend that the data from this study follows.

The role of light intensity (lx) and its interaction with colour did not appear to be important in survival of larval prawn. This agrees with Rodrigues and colleagues (1998) who reported that light intensities ranging from 250 to 2500 lx yielded good survival. In this study all tanks were exposed to identical light sources, but showed varying light intensities, because of the different reflective values of the various colours. However, no significant correlation ($P > 0.05$) between light intensity and survival was indicated (Fig. 1). In conclusion, tank colour had no significant impact on metamorphosis rate, growth or time to metamorphosis. However, survival was significantly ($P > 0.05$) impacted by tank colour.

These data indicate that tank colour plays a significant role in the survival of larval prawns grown in indoor recirculating systems. Based on these data, the use of red and green tanks is recommended, while white or blue tanks are not suggested. Further research avenues might include evaluating different hues of the best performing tank colours, the use of coloured lighting and its effect on the ability of the larval prawns to capture and consume food items, and the identification of critical periods in larval development.

This data may allow commercial prawn hatcheries to increase survival and decrease hatchery produc-

tion costs, which could translate to increased profitability for commercial growout facilities.

Acknowledgments

The authors thank Aaron VanArnum, Miles Lange, Charles Weibel and Leigh Anne Bright for assisting in stocking, harvesting, and collecting data from this experiment. This project was funded by The Regional University Excellence Trust Fund.

References

Aquacop (1977) *Macrobrachium rosenbergii* (De Man) culture in Polynesia: progress in developing a mass intensive larval rearing technique in clear water. *Proceedings of the World Mariculture Society* **8**, 311–319.

Aquacop (1983) Intensive larval rearing in clear water of *Macrobrachium rosenbergii* (De Man) at the Centre Oceanologique du Pacifique, Tahiti. In: *CRC Handbook of Mariculture, Vol. 1: Crustacean Aquaculture* (ed. by J.P. McVey & J.R. Moore), pp. 179–187. CRC Press, Boca Raton, FL, USA.

Armstrong D.A., Stephenson M.J. & Knight A.W. (1976) Acute toxicity of nitrite to larvae of giant Malaysian prawn *Macrobrachium rosenbergii*. *Aquaculture* **9**, 39–46.

Barton B.A. & Iwama G.K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Disease* **1**, 3–26.

Boyd C.E. (1979) *Water Quality in Ponds for Aquaculture*. Alabama Agricultural Experiment Station, Auburn University, Auburn, AL, USA.

D'Abramo L.R., Daniels W.H., Fondren M.W. & Brunson M.W. (1995) *Management practices for the culture of freshwater prawn Macrobrachium rosenbergii in temperate climates*. Bulletin 1030, Mississippi Agricultural Forestry Experimental Station, Mississippi State University, Mississippi, USA.

Daniels W.H., D'Abramo L.R. & Parseval L.D. (1992) Design and management of a closed recirculating 'clearwater' hatchery system for freshwater prawns *Macrobrachium rosenbergii*. *Journal of Shellfish Research* **11**, 65–73.

Dasgupta S. & Tidwell J.H. (2003) A breakeven price analysis of four hypothetical freshwater prawn, *Macrobrachium rosenbergii*, farms using data from Kentucky. *Journal of Applied Aquaculture* **14**, 1–22.

Fanta E. (1995) Influence of background colour on the behaviour of the fish *Oreochromis niloticus*. *Arquivos de Biologia eTechnolgia* **384**, 1237–12251.

Juarez L.M., Holtschmit K.H., Salmeron J.J. & Smith K.M. (1987) The effects of chemical and visual communication, space availability, and substratum colour on the growth of juvenile freshwater prawn *Macrobrachium rosenbergii*. *Journal of Experimental Marine Biology and Ecology* **110**, 285–295.

Lin X. & Omori M. (1993) Effect of tank colouration on the feeding rates of zoeal larvae of the giant freshwater shrimp *Macrobrachium rosenbergii*. *Bulletin of the Plankton Society of Japan* **40**, 19–25.

Manzi J.J., Maddox M.B. & Sandifer P.A. (1977) Algal supplement enhancement of *Macrobrachium rosenbergii* larviculture. *Proceedings of the World Mariculture Society* **8**, 207–223.

Moller T.H. (1978) Feeding behaviour of larvae and post larvae of *Macrobrachium rosenbergii*. *Journal of Experimental Marine Biology and Ecology* **35**, 251–258.

Ostrowski A.C. (1989) Effect of rearing tank background colour on early survival of dolphin larvae *Coryphaena hippurus*. *The Progressive Fish Culturist* **51**, 161–163.

Papoutsoglou S.E., Mylonakis G., Miliou H., Karakatsouli N.P. & Chadio S. (2000) Effect of background colour on growth performances and physiological responses of scaled carp *Cyprinus carpio* reared in a closed circulated system. *Aquacultural Engineering* **22**, 309–318.

Rodrigues C.C.B., Rodrigues J.B.R. & Moschen S. (1998) The effects of sunlight, and tank colour on the development and post-larvae production of *Macrobrachium rosenbergii*. *Anais do Aquacultura Brazil* **2**, 423–435.

Uno Y. & Kwon C.S. (1969) Larval development of *Macrobrachium rosenbergii* reared in the laboratory. *Journal of the Tokyo University of Fisheries* **55**, 179–190.

Valenti W.C., Mallasen M. & Silva C.A. (1998) Larvicultura em sistema fechado dinamico. In: *Carcinicultura de Agua Doce: Technologia para a Producao de Camaroes* (ed. by W.C. Valenti), pp. 112–139. IBAMA, Brasilia, Brazil.

Woods T., Murdock J. & Riggins S. (1998) Freshwater shrimp enterprise costs, and returns estimates for Kentucky. *Agriculture Economics Extension Publication* **1**, 98–105.

Zar J.H. (1989) *Biostatistical Analysis*, 4th edn. Prentice-Hall, Upper Saddle River, NJ, USA.

Zimmermann S. (1998) Manejo da fase de crescimento final. In: *Carcinicultura de Agua Doce: Technologia para a Producao de Camaroes* (ed. by W.C. Valenti), pp. 191–215. IBAMA, Brasilia, Brazil.