Improved techniques for rearing mud crab *Scylla paramamosain* (Estampador 1949) larvae

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**Abstract**

A series of rearing trials in small 1 L conical and large tanks of 30–100 L were carried out to develop optimal rearing techniques for mud crab (*Scylla paramamosain*) larvae. Using water exchange (discontinuous partial water renewal or continuous treatment through biofiltration) and micro-algae (*Chlorella* or *Chaetoceros*) supplementation (daily supplementation at 0.1–0.2 million cells mL⁻¹ or maintenance at 1–2 million cells mL⁻¹), six different types of rearing systems were tried. The combination of a green-water batch system for early stages and a recirculating system with micro-algae supplementation for later stages resulted in the best overall performance of the crab larvae. No clear effects of crab stocking density (50–200 larvae L⁻¹) and rotifer (30–60 rotifers mL⁻¹) and *Artemia* density (30–200 L⁻¹) were observed. A stocking density of 100–150 zoeae 1 (Z1) L⁻¹, combined with rotifer of 30–45 mL⁻¹ for early stages and *Artemia* feeding at 10–15 nauplii mL⁻¹ for 23–25 seemed to produce the best performance of *S. paramamosain* larvae. Optimal rations for crab larvae should, however, be adjusted depending on the species, larval stage, larval status, prey size, rearing system and techniques. A practical feeding schedule could be to increase live food density from 30 to 45 rotifers mL⁻¹ from Z1 to Z2 and increase the number of *Artemia* nauplii mL⁻¹ from 10 to 15 from Z3 to Z5. Bacterial disease remains one of the key factors underlying the high mortality in the zoea stages. Further research to develop safe prophylactic treatments is therefore warranted. Combined with proper live food enrichment techniques, application of these findings has sustained a survival rate from Z1 to crab 1–2 stages in large rearing tanks of 10–15% (maximum 30%).

**Keywords:** *Scylla paramamosain*, rearing techniques, water exchange, micro-algae, larval density, live food density, prophylaxis

**Introduction**

Aquaculture of mud crabs, *Scylla* spp., contributes a large proportion to the world production of the genus (FAO1999). Moreover, mud crabs represent a valuable component of small-scaled coastal fisheries in many countries in tropical and subtropical Asia, for which there has been a general trend of increased exploitation in recent years (Angell 1992; Keenan 1999a). In Vietnam, the mud crab *Scylla paramamosain* is the second most important marine species next to shrimp, being cultured widely in the coastal area. However, mud crab farming currently relies entirely on the wild for seed stock and the main obstacle for expansion is the unavailability of hatchery-reared seed (Liong 1992; Rattanachote & Dangwatanakul 1992; Keenan 1999a; Shelley & Field 1999; Mann, Asakawa, Pizzuto, Keenan & Brock 2001; Xuan 2001).

Rearing techniques, disease and nutrition are the three main areas of research that have supported commercial production of marine fish and crustacean larvae (Sorgeloos & Legé 1992). These three aspects are to a large extent interconnected and developing hatchery techniques for a new species is not possible unless all the areas are addressed. The
design of rearing systems covers more than purely
technical aspects. Sub-optimal rearing conditions
(e.g. physical stress, lack of oxygen or sub-optimal
water quality) affect larval health and can cause
mass mortality due to disease outbreaks. Similarly
system design influences (live) food quality and its
availability to the predator larvae.

There has been a great deal of progress in marine
larval rearing technology since its beginning in the
1960s (Shelbourne 1964; Howell, Day, Ellis & Baynes
1998). Many of these technical improvements developed
over the past decades could be applied for mud
crab with some modifications. An overview of the
rearing systems currently applied for larviculture of
mud crabs was presented by Davis (2003). Although
knowledge has been obtained from these systems,
there is a need to further optimize rearing techniques
in order to maximize larval survival and quality.
Furthermore, techniques should be adapted for each
species (Keenan, Davis & Mann 1998; Keenan
1999b) in relation to local conditions (seawater
source, status of hatchery management, local
resources). The aim of this research is to adapt the
existing rearing systems for larviculture to mud crab
species (S. paramamosain being the test case) and to
improve other techniques in order to maximize larval
survival and quality.

Materials and methods

Source of larvae

Grown crabs were bought from local markets and
transported to the hatchery. Before stocking in the
hatchery, the crabs were subjected to a bath of
100 μL L⁻¹ of a 40% formalin solution for 1 h. The
crabs were stocked individually in 100 L compartments
of a roofed 2 x 2 x 0.5 m cement tank, equipped with a biofilter. Rearing water of
30 ± 1 g L⁻¹ salinity was diluted from brine (90-
130 g L⁻¹) with tap water and chlorinated before use.
Ambient temperature fluctuated slightly around
28 °C. Every crab was fed a daily ration of 10–15 g of
fresh marine squid, herring or shrimp meat alternately.

After 3–5 days of acclimation, unilateral eyestalk
ablation was applied to induce spawning. After spawning, berried crabs were again subjected to a
100 μL L⁻¹ formalin bath for 1 h and transferred to a
70 L plastic tank connected to a biofilter for egg
incubation. Daily management consisted of siphoning out
waste materials and shedding eggs from the tank
bottom and controlling the temperature (30 °C), salinity
(30 g L⁻¹) and ammonium and nitrite levels. Every
other day, the crabs were placed in a 50 μL L⁻¹ for­
malin bath for 1 h to reduce or prevent infestation of
the eggs with fungi and bacteria. During egg incubation, the crabs were not fed.

One to two days before hatching, the berried
female was moved to a 500 L fiberglass tank. When
the hatching process was completed, larvae were
selected based on their phototactic behavior. Aeration
in the hatching tank was turned off for several
minutes and the active larvae swimming up to the
surface were collected by gentle scooping.

The larvae were then transferred to the rearing
containers. Acclimation was performed by placing the
larvae in a 50 L plastic mesh bucket and slowly
rinsing them with water from the larval rearing con­
tainers for 20–30 min before release.

Food and feeding

Micro-algae culture

Starting cultures of the micro-algae Chaetoceros calcitrans and Chlorella vulgaris were maintained indoors
with Valine solution in seawater of 30 g L⁻¹ at 25 °C.
Large-scale production was performed indoors in
500 L tanks under a transparent roof. A haemocytom­
eter was used to count micro-algal densities.

Rotifer culture and enrichment

The same Brachionus plicatilis L strain with a loran
length and width of 164 ± 22 and 120 ± 22 μm,
respectively, was used in all the experiments. Rotifiers
were cultured indoors in 1000 L fiberglass tanks oper­
ad in a batch mode following the procedure de­
scribed in Sorgeloos and Lavens (1996). Rotifiers
were initially grown on baker's yeast, but later on led
culture Selco® (INVE Aquaculture, Dendermonde
Belgium) before feeding the larvae. Temperature
and salinity were controlled at 25 °C and 25 g L⁻¹
respectively. They were harvested through a 60 μm
screen and rinsed thoroughly.

Rotifiers were enriched with micro-algae or artificial
enrichment media before being fed to the crab
larvae. Enrichment with Chlorella was performed at
a density of 5 x 10⁶ cells mL⁻¹ for 3 h (Dhert 1996).
Rotifiers were also enriched with Dry Immune Selco®
(DIS®, INVE Aquaculture), using two separate doses of
0.05 g L⁻¹ at a 3-h interval. Enrichment was per­
formed at a density of 500 rotifers mL⁻¹. The water
in the enrichment vessel was slowly heated to
29–30 °C to avoid exposing the rotifers to thermal shock when they were added to the larval rearing tanks. Before being fed to the larva, enriched rotifers were rinsed and re-suspended in clean seawater at the same temperature as the crab-rearing tanks.

**Artemia culture and enrichment**

*Artemia* nauplii (Vincent Chau strain) were hatched as described by Van Stappen (1996). Both newly hatched or enriched *Artemia* nauplii were used in the experiments of this study. *Artemia* were enriched with Chlorella at the same micro-algal density as for rotifer enrichment. The nauplii were also enriched with 

In experiments 4–8, other culture aspects such as 21 stocking density, live food density and the effect of different prophylactic treatments were investigated. The water quality management schemes tested in experiments 1–3 are summarized in Table 1. An overview of the experimental design and culture conditions of all the experiments is presented in Table 2. The small-scale experiments (1–3) were carried out in a temperature-controlled room (28–30 °C). The experiments in 100 L tanks were performed outdoors at ambient temperature (27–31 °C). The source and the disinfection procedure of the seawater for larval rearing was similar to those used for broodstock rearing. Formalin at a concentration of 20 µL L⁻¹ was applied every other day as a prophylactic treatment in experiments 1–6.

**Larval rearing experiments: objectives and experimental design**

In experiments 1, 2 and 3, the effect of different water exchange schemes and the addition of micro-algae on larval survival and development were evaluated. In experiments 4–8, other culture aspects such as 21 stocking density, live food density and the effect of different prophylactic treatments were investigated.

**Table 1 Overview of larval rearing systems applied in this study based on the method of water exchange and micro-algae supplementation**

<table>
<thead>
<tr>
<th>Algae supplementation</th>
<th>Water exchange</th>
<th>Continuous water treatment through the use of a biofilter</th>
</tr>
</thead>
<tbody>
<tr>
<td>No micro-algae supplemented (indoors)</td>
<td>Clear-Batch system</td>
<td>Clean-Recirc system</td>
</tr>
<tr>
<td>Micro-algae supplemented at low levels to provide extra food for live prey (indoors or outdoors)</td>
<td>Algae-Batch system</td>
<td>Algae-Recirc system</td>
</tr>
<tr>
<td>Micro-algae supplemented at a high concentration and self-sustainable under natural sunlight as an extra food for live prey and water conditioning (outdoors)</td>
<td>Green-Batch system</td>
<td>Green-Recirc system (Combination of Green-Recirc and Algae-Recirc system at early and late larval stages, respectively)</td>
</tr>
</tbody>
</table>

Experiment 1

Larval survival and growth in a clear water system with daily partial water exchange (Clear-Batch) was compared with those in a clear water recirculating system (Clear-Recirc). In the first rearing system, 30–50% of the water was replaced daily. In the recirculating system, all rearing tanks were connected to a central biofilter. Water was recirculated at a rate of approximately 100% of the tank volume every 3–4 h. Live food and crab larvae were retained in the rearing tanks with a mesh screen of 70 and 300 µm during the rotifer and Artemia feeding stage, respectively. Larger mesh screens (250 and 500–1000 µm for rotifer and Artemia stage, respectively) and higher flow
Table 2 Overview of the experimental conditions and water quality parameters (mean ± standard deviation) in experiments 1–8

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Factor</th>
<th>Rearing system</th>
<th>Container volume (L)</th>
<th>No. of replicates</th>
<th>Stocking density (Z1/L·L⁻¹)</th>
<th>Ammonium (mg L⁻¹)</th>
<th>Nitrates (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rearing system</td>
<td>Clean-Batch</td>
<td>30</td>
<td>5</td>
<td>50</td>
<td>0.35 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Rearing system</td>
<td>Clean-Batch</td>
<td>100</td>
<td>8</td>
<td>100</td>
<td>0.32 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Rearing system</td>
<td>Green-Batch</td>
<td>100</td>
<td>4</td>
<td>100</td>
<td>1.54 ± 1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Z1 density</td>
<td>Green-Batch</td>
<td>100</td>
<td>3</td>
<td>50</td>
<td>1.50 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53 ± 0.23&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Rotifer density (30, 45 and 50 rotifers/mL&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Clear-Recirc</td>
<td>30</td>
<td>5</td>
<td>50</td>
<td>1.07 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43 ± 0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>Artemia density (10, 15 and 20 Artemias/L&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Clear-Recirc</td>
<td>30</td>
<td>5</td>
<td>100</td>
<td>0.04 ± 0.05</td>
<td>0.13 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>Propriolids treatment (control, formalin and oxytetracycline)</td>
<td>Clear-Recirc</td>
<td>4</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>Duration of direct ozonation (control: without ozonation and ozonation for 2, 4, 6, 8 and 10 min)</td>
<td>Clear-Batch</td>
<td>1</td>
<td>2</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup>Cylindrical mesh tanks of 30–100 L and plastic cases of 1 L. 
<sup>b</sup>Values within an experiment in the same column followed by the same superscript letter are not statistically different (P ≥ 0.05). 
For a description of rearing systems refer to Table 1. 
ND, not determined.
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rates were used upon daily flushing out of uneaten live food and waste.

Experiment 2

A Clear-Recirc system was compared with two systems where micro-algae were added. Rearing conditions for the Clear-Recirc system were similar to those described in experiment 1. In the Algae-Recirc system, micro-algae were added daily to the recirculating system at a low concentration ranging from 0.1 to 0.2 million cells mL\(^{-1}\). The operation of the rearing tanks was similar to the Clear-Recirc treatment. In the Green-Batch treatment, a classical green-water system; micro-algae concentrations in the culture tanks were kept at a tenfold higher level of 1–2 million cells mL\(^{-1}\). In this system, the culture tanks were initially only filled to 30% of their capacity and gradually increased to 100% by the end of the Z2 stage by adding water and algae daily. Later on, 30–50% of the rearing water was replaced daily by clean seawater and/or algae, depending on the density of micro-algae remaining in the rearing tanks. Upon water exchange, uneaten live food was also flushed out through a mesh screen (mesh sizes as described in experiment 1). The same amount of live food (30–45 rotifers mL\(^{-1}\) and 5–30 Artemia nauplii mL\(^{-1}\)) was fed in all the treatments. In the systems using algae, Chlorella was used for Z1–Z3 stages (which is unsuitable as a food source for Artemia) from Z4 onwards. Chlorella was gradually replaced with Chaetoceros.

Experiment 3

In this experiment, a Green-Batch and a Green-Recirc system were set up in order to further evaluate the application of micro-algae on the performance of crab larvae. The first rearing system was a batch system with addition of high concentrations of algae as described in experiment 2. The second system consisted of a combination of the Green-Batch system for early crab stages (Z1–Z2) and a Algae-Recirc system for later stages (Z3 onwards).

Experiments 4–6

In these experiments, the effect of Z1 stocking density (50, 100, 150 and 200 L\(^{-1}\), experiment 4), rotifer feeding densities at 30, 45 and 60 mL\(^{-1}\) for Z1–Z2 (experiment 5) and Artemia densities at 10, 15 and 20 mL\(^{-1}\) for Z3 onwards (experiment 6) was evaluated. These experiments were run in a Green-Batch (experiment 4) or a Clear-Recirc system (experiments 5 and 6) as described above.

Experiment 7

In experiment 7, the effect of prophylactic chemicals on the survival of the larvae was investigated. Three treatments, consisting of a control (no chemicals used), daily addition of formalin at 20 \(\mu\)L L\(^{-1}\) and daily addition of oxytetracycline at 10 mg L\(^{-1}\), were run in 1 L plastic cones. All cones were placed in a water bath in order to maintain the rearing temperature at 30 °C. Water was replaced almost completely daily upon water exchange; the survival was determined.

Experiment 8

To avoid the use of drugs as a prophylaxis, direct consumption of the culture tanks was tested in aerated 1 L plastic cones. Ozone \((O_3)\) was injected directly via an airstone into every larval rearing cone upon changing water and feed daily. Six treatments with three replicates were arranged consisting of a control (without \(O_3\) application) and \(O_3\) injection for 2, 4, 6, 8 and 10 min (equivalent to a residual \(O_3\) level in the water of 0.06, 0.12, 0.15, 0.17 and 0.19 mg L\(^{-1}\) as measured by a test kit upon finishing the injection). Other rearing conditions and daily management were similar to those described for experiment 7.

Evaluation criteria

The survival rates in the experiments using large (30–100 L) containers (experiments 1–6) were estimated by volumetric sampling. Depending on the tank volume and the density of the surviving larvae, triplicate 300–1000 mL samples were taken from each tank. Megalopae (M) (DAH 15–18) and first crabs (CI) (DAH 22) were counted individually. In experiments 7 and 8 (using small cones), the average survival rate was calculated by individually counting all surviving larvae in each replicate.

Larval development was monitored every 3 days but daily in experiments 7 and 8 by identifying the average zoeal instar stage of a sample of larvae and assigning it a value: Z1 = 1, Z2 = 2, etc. Megalopa stage was assigned a value of 6. To compare the larval development in each treatment, an average larval stage index (LSI) was calculated from the average LSI value of all replicate containers in the same treatment. For large containers (experiments 1–6), five or 10 larvae (in 30 and 100 L tanks respectively) were
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Table 3: Experiment 1: survival rates and larval stage index (LSI) values of Scylla paramamosain larvae cultured in two different rearing systems

<table>
<thead>
<tr>
<th>Days after hatch</th>
<th>Treatment</th>
<th>Survival rate (%)</th>
<th>LSI'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clear-Batch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>85 ± 6a</td>
<td>1.5 ± 0.2a</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>78 ± 6a</td>
<td>2.7 ± 0.1a</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>70 ± 6a</td>
<td>3.5 ± 0.4a</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>64 ± 7a</td>
<td>4.0 ± 0.0a</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>42 ± 6b</td>
<td>4.6 ± 0.2a</td>
<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>32 ± 5b</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clear-Recirc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>84 ± 4a</td>
<td>1.5 ± 0.2a</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>78 ± 6a</td>
<td>2.7 ± 0.1a</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>70 ± 5a</td>
<td>3.8 ± 0.3a</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>63 ± 9a</td>
<td>4.2 ± 0.3a</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>47 ± 6a</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

'Survival rates or LSI values in the same column followed by the same superscript letter are not statistically different (P > 0.05).

For treatment descriptions, refer to Table 1.
ND, not determined.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare data. Homogeneity of variance was tested with the Levene statistic (F or Z value was set at 0.05). If no significant differences were detected between the variances, the data were submitted to a one-way ANOVA. Tukey's honestly significant difference post hoc analysis was used to detect differences between means and to indicate areas of significant difference. If significant differences were detected between variances, data were transformed using the arcsine-square root (for percentage data, i.e. survival rate) or logarithmic transformations (for LSI value) (Sokal & Rohlf 1995). All analyses were performed using the statistical program STATISTICA 6.0.

Results

Experiment 1

Survival in the Clear-Recirc system at 74–75 stages on DAH 15 and in the megalop stage on DAH 18 was significantly higher than those in the Clear-Batch system (both at P < 0.01) (Table 3). Although slightly higher in the recirculating system, LSI was not significantly different between treatments. The better larval performance in the Clear-Recirc system was accompanied by significantly lower average ammonia levels (P < 0.01) and slightly lower nitrite levels (see Table 2).

Experiment 2

On DAH 9, larval survival in the Clear-Recirc system was significantly lower (P < 0.05) than in both treatments with micro-algae supplementation (Algae-Recirc and Green-Batch systems) (Table 4). On DAH 12, survival in the Clear-Recirc treatment was lower (P < 0.05) than in the Algae-Recirc system, whereas the Green-Batch system had intermediate results. The LSI values on DAH 15 show a similar trend, although not significantly different.

The average levels of ammonia and nitrite in the Clear-Recirc and Algae-Recirc systems were significantly lower (P < 0.01) than those in the Green-Batch system (see Table 2). In the Green-Batch system, peaks of ammonia and nitrite concentrations of 3 and 1 mg L⁻¹, respectively, were recorded at the end of the experiment.

Experiment 3

Table 5 presents the larval performance of the crab larvae cultured in two rearing systems. The survival rates and LSI values of both rearing systems were not significantly different. However, the survival rates on later days (from DAH 12–22) in the Green-Recirc system tended to be higher than those in the Green-Batch system. The biofilter had a positive impact on
Table 4: Experiment 2: survival rates and larval stage index (LSI) values of *Scylla paramamosain* larvae cultured in three different rearing systems.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after hatch</th>
<th>Survival rate (%)*</th>
<th>LSI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Clear-Recirc</td>
<td>74 ± 12a</td>
<td>63 ± 7a</td>
<td>44 ± 6a</td>
</tr>
<tr>
<td>Algae-Recirc</td>
<td>74 ± 12a</td>
<td>63 ± 9a</td>
<td>61 ± 7a</td>
</tr>
<tr>
<td>Green-Batch</td>
<td>74 ± 11a</td>
<td>67 ± 2a</td>
<td>58 ± 9a</td>
</tr>
</tbody>
</table>

*Survival rates or LSI values in the same column followed by the same superscript letter are not statistically different (P > 0.05). For treatment descriptions, refer to Table 1.

Table 5: Experiment 3: survival rates and larval stage index (LSI) values of *Scylla paramamosain* larvae cultured in two different rearing systems.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after hatch</th>
<th>Survival rate (%)*</th>
<th>LSI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Green-Batch</td>
<td>94 ± 6a</td>
<td>88 ± 9a</td>
<td>80 ± 2a</td>
</tr>
<tr>
<td>Green-Recirc</td>
<td>94 ± 6a</td>
<td>88 ± 9a</td>
<td>80 ± 2a</td>
</tr>
<tr>
<td>LSI*</td>
<td>Green-Batch</td>
<td>1.4 ± 0.3a</td>
<td>2.7 ± 0.1a</td>
</tr>
<tr>
<td></td>
<td>Green-Recirc</td>
<td>1.4 ± 0.3a</td>
<td>2.6 ± 0.2a</td>
</tr>
</tbody>
</table>

*Survival rates or LSI values in the same column followed by the same superscript letter are not statistically different (P > 0.05). For treatment descriptions, refer to Table 1.

ND, not determined.

Experiment 4

Table 6 shows the survival and development rate of crab larvae stocked at four different Z1 densities (50, 100, 150 and 200 L⁻¹). The survival rates were not significantly different among treatments. Only on DAH 6 was a negative correlation between LSI and larval density observed.

Experiment 5

Table 7 shows the survival rates and the LSI values of crab larvae fed three different rotifer densities in the Z1–Z2 stages. No significant differences were found for any of the parameters. It can, however, be noticed that LSI was always the lowest in treatment 30 rotifiers L⁻¹ and the highest in treatment 60 rotifiers L⁻¹.

Experiment 6

Table 8 presents the survival and development rate of crab larvae fed *Artemia nauplii* at three densities (10, 15 and 20 mL⁻¹) from the Z3 stage onwards. No significant differences were observed between the treatments. There seemed, however, to be a weak trend towards higher survival and LSI with increasing *Artemia* density towards the end of the trial.

Experiment 7

Table 9 shows the survival and development rate to the megalopa stage (DAH 22) of larvae receiving different prophylactic treatments. From DAH 6 onwards, the survival rate of larvae in the treatment
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Table 6  Experiment 4: survival rates and larval stage index (LSI) values of Scylla paramamosain larvae stocked at four different 21 densities (21L⁻¹)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after hatch</th>
<th>Survival rate (%)*</th>
<th>LSI*</th>
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<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>50</td>
<td>79 ± 2a</td>
<td>56 ± 19a</td>
<td>42 ± 16a</td>
</tr>
<tr>
<td>100</td>
<td>80 ± 7a</td>
<td>74 ± 4a</td>
<td>71 ± 10a</td>
</tr>
<tr>
<td>150</td>
<td>79 ± 2a</td>
<td>57 ± 12a</td>
<td>45 ± 9a</td>
</tr>
<tr>
<td>200</td>
<td>55 ± 5a</td>
<td>53 ± 17a</td>
<td>42 ± 8a</td>
</tr>
</tbody>
</table>

*Survival rates or LSI values in the same column followed by the same superscript letter are not statistically different (P > 0.05).

ND, not determined.

Table 7  Experiment 5: survival rates and larval stage index (LSI) values of Scylla paramamosain larvae fed three different rotifer densities (rotifers mL⁻¹) from day 0 to day 6 after hatch

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after hatch</th>
<th>Survival rate (%)*</th>
<th>LSI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>30</td>
<td>69 ± 7a</td>
<td>53 ± 10a</td>
<td>30 ± 8a</td>
</tr>
<tr>
<td>45</td>
<td>87 ± 6a</td>
<td>58 ± 6a</td>
<td>35 ± 7a</td>
</tr>
<tr>
<td>60</td>
<td>87 ± 5a</td>
<td>55 ± 7a</td>
<td>32 ± 6a</td>
</tr>
<tr>
<td>LSI*</td>
<td>30</td>
<td>1.8 ± 0.2a</td>
<td>2.7 ± 0.1a</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>1.8 ± 0.2a</td>
<td>2.8 ± 0.1a</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.8 ± 0.2a</td>
<td>2.8 ± 0.2a</td>
</tr>
</tbody>
</table>

*Survival rates or LSI values in the same column followed by the same superscript letter are not statistically different (P > 0.05).

Discussion

Rearing system

Recirculation

Water recirculation through a biofilter in the Clear-Recirc system positively affected larval performance compared with manual partial water replacement in the Clear-Batch system (experiment 1). The advantages of recirculating systems in commercial fish and crustacean larval production have been proven before for other species. Research into recirculating...
Table 8  
**Experiment 6: survival rates and larval stage index (LSI) values of* Scylla paramamosain* larvae-fed three different instar-1 *Artemia* densities (*Artemia ml⁻¹*) from 6 days after hatch**  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after hatch</th>
<th>Survival rate (%)*</th>
<th>LSI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>26 ± 3a</td>
<td>12 ± 5a</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>15</td>
<td>30 ± 6a</td>
<td>15 ± 7a</td>
<td>10 ± 6a</td>
</tr>
<tr>
<td>20</td>
<td>32 ± 8a</td>
<td>16 ± 9a</td>
<td>18 ± 9a</td>
</tr>
<tr>
<td>LSI*</td>
<td>10</td>
<td>3.1 ± 0.2°</td>
<td>3.7 ± 0.4a</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3.1 ± 0.1a</td>
<td>3.7 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.2 ± 0.1°</td>
<td>3.8 ± 0.3a</td>
</tr>
</tbody>
</table>

*Survival rates or LSI values in the same column followed by the same superscript letter are not statistically different (P > 0.05).

Table 9  
**Experiment 7: survival rates and larval stage index (LSI) values of* Scylla paramamosain* larvae treated daily with prophylactic chemicals**  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after hatch</th>
<th>Survival rates (%)*</th>
<th>LSI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Control</td>
<td>85 ± 3a</td>
<td>64 ± 7°</td>
<td>48 ± 8°</td>
</tr>
<tr>
<td></td>
<td>54 ± 10b</td>
<td>28 ± 11b</td>
<td>17 ± 7°</td>
</tr>
<tr>
<td>10</td>
<td>9 ± 6°</td>
<td>11 ± 6°</td>
<td>7 ± 5°</td>
</tr>
<tr>
<td>Formalin</td>
<td>84 ± 7°</td>
<td>60 ± 6b</td>
<td>47 ± 7°</td>
</tr>
<tr>
<td></td>
<td>34 ± 12b</td>
<td>26 ± 6b</td>
<td>13 ± 10b</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>91 ± 4a</td>
<td>80 ± 2a</td>
<td>74 ± 4°</td>
</tr>
<tr>
<td></td>
<td>52 ± 6a</td>
<td>34 ± 3°</td>
<td>24 ± 5°</td>
</tr>
<tr>
<td>LSI*</td>
<td>1.8 ± 0.1°</td>
<td>2.6 ± 0.2°</td>
<td>3.4 ± 0.3°</td>
</tr>
<tr>
<td></td>
<td>4.5 ± 0.3°</td>
<td>5.1 ± 0.1°</td>
<td>5.5 ± 0.1°</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.1°</td>
<td>3.7 ± 0.1°</td>
<td>4.2 ± 0.3°</td>
</tr>
<tr>
<td></td>
<td>5.1 ± 0.1°</td>
<td>5.8 ± 0.1°</td>
<td>6.0 ± 0.1°</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.1°</td>
<td>3.3 ± 0.2°</td>
<td>4.2 ± 0.1°</td>
</tr>
<tr>
<td></td>
<td>4.5 ± 0.1°</td>
<td>6.3 ± 0.2°</td>
<td>5.6 ± 0.2°</td>
</tr>
</tbody>
</table>

*Survival rates or LSI values in the same column followed by the same superscript letter are not statistically different (P > 0.05).

Table 10  
**Experiment 8: survival rates of* Scylla paramamosain* larvae treated daily by ozone for different durations of time (min)**  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DAH 3 (22)</th>
<th>DAH 6 (23)</th>
<th>DAH 9 (24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78 ± 6a</td>
<td>25 ± 9b</td>
<td>9 ± 6a</td>
</tr>
<tr>
<td>Ozone2</td>
<td>67 ± 8b</td>
<td>52 ± 14a</td>
<td>11 ± 10a</td>
</tr>
<tr>
<td>Ozone4</td>
<td>60 ± 17m</td>
<td>33 ± 11a</td>
<td>5 ± 2a</td>
</tr>
<tr>
<td>Ozone6</td>
<td>45 ± 25m</td>
<td>27 ± 12b</td>
<td>5 ± 3a</td>
</tr>
<tr>
<td>Ozone8</td>
<td>31 ± 28a</td>
<td>19 ± 8a</td>
<td>0 ± 0a</td>
</tr>
<tr>
<td>Ozone10</td>
<td>24 ± 15°</td>
<td>12 ± 9a</td>
<td>0 ± 1a</td>
</tr>
</tbody>
</table>

Survival rates in the same column followed by the same superscript letter are not statistically different (P > 0.05).  
DAH, days after hatch; 2, ozone. Control, without ozone; Ozone1, 2, 4, 6, 8, and 10 duration of ozone injection from 2 to 10 min, which is equivalent to 0.06, 0.12, 0.15, 0.17 and 0.19 mg L⁻¹ of the residual ozone respectively.

systems has also been identified as a priority for shrimp culture (Lawrence & Lee 1997). In these systems, water exchange is minimized through the use of biological, chemical and/or mechanical filtration to maintain good water quality continuously. As they provide less stress and confer constant good water quality to the larvae, these systems are able to maintain a high biological carrying capacity in relatively little space (Quilliere, Marie, Roux, Gosse & Moret- 
gauldry 1993, Twarowiska, Westerman & Lascord 1997). For crab aquaculture, recirculating systems also appear to warrant further investigation in order to decrease labour requirements and seawater consumption, providing a more stable culture medium.
and thus reducing larval stress. If the system design is kept simple, recirculating systems could also be suitable for large-scale production.

**Role of supplemented micro-algae**

The addition of micro-algae to the recirculation systems resulted in both higher survival and faster development in this study. Micro-algae have been proven to be beneficial by various modes of action. They could help maintain the quality of live food. As in the culture of marine fish larvae, unconsumed rotifers may reside in the tanks for several days and their nutritional value may become severely reduced (Makridis & Olsen 1999). Furthermore, according to these authors, poorly fed rotifers were more sensitive to starvation than well-fed rotifers, as their nitrogen content decreased at a higher rate.

Micro-algae also play an important role in stabilizing water quality via either ammonia uptake or oxygen production (Tang, Huang & Liao 1991). Because the Clear-Recirc system already provided optimal water quality, it is unlikely that the stabilizing effect on water quality is responsible for the improved performance in the algae-supplemented system. In batch culture systems, this effect would probably be much more pronounced. A direct comparison between a green and clear water batch system was, however, not made in this study.

In a study on the effect of *Chlorella* on the population of luminescent bacteria *Vibrio harveyi*, no luminescent bacteria were recovered on days 2 and 3 in flasks with *Chlorella*, while those without the micro-algae still harboured luminescent bacteria at day 3 (Tendencia & dela Peña 2003). Also, the diatom *Chaetoceros* has been shown to produce natural antibiotics and high concentrations of this marine diatom will eliminate *Vibrio vulnificus* and other pathogenic bacteria, which contribute to the propagation of viruses in the shrimp production environment (Wang 2003).

In conclusion, micro-algae in mud crab larval rearing may play a role in improving and maintaining live food quality and controlling bacteria levels.

**Choice of system**

In experiment 3, the Green-Recirc system (which is a combination of a Green-Batch system during the rotifer feeding stage and a Algae-Recirc system thereafter) seemed to be better than the Green-Batch system. The Green-Batch system seems to be more appropriate for early stages of crab larva (Z1–Z2) as it is less stressful for the early stages and easier to gradually fill up the tanks with fresh seawater, algae and rotifers than flushing out old rotifers in the recirculation system. In the recirculating system, the young larvae may be prone to physical damage and may spend considerable energy trying to swim up against the current. Early crab larvae are delicate due to their small size and the three long spines on the carapace that are easily damaged when they are entrapped on the mesh screen during flushing out of uneaten food in the recirculation system (Davis 2003). The nutritional effect of micro-algae is probably also more pronounced during the rotifer feeding stage than during the Artemia feeding stage. Furthermore, it is not necessary to recirculate water during these first days, as the concentrations of ammonia and nitrate are still low. Using the Algae-Recirc system in later stages is more favourable for reducing the increasing ammonia and nitrate concentrations as no waste material is produced by the crab larvae. Moreover, as the larvae develop into more efficient predators, feed is consumed faster, and maintenance of optimal food quality is less of an issue. Many studies successfully applied a similar combined rearing technique due to its benefit for the larvae and convenience for management, particularly for large rearing containers. Under green-water culture conditions, water is not exchanged for the first 3 days. Thereafter, water exchange is slowly increased from 10%–20% day⁻¹ for 22–23 to between 40% and 50% day⁻¹ at the end of the rearing cycle (Z4–M) (Männ, Asakawa & Pitazzu 1999; Quirito, Pardeo-Extpea, Millamena, Rodriguez & Bolognani 2003). In Japan, a mesocosm system is used for culturing larvae in larger tanks (> 10 m³). The tanks are partially filled with green water at Z1 (20–35% volume), tanks are then filled up with clean seawater during the course of the Z2–Z3 stages and during the Z4 and M stages water is exchanged on a flow-to-waste basis (Hamesaki, Suprayudi & Takeuchi 2002).

**Other rearing techniques**

**Z1 stocking density**

No significant effect of larval density was observed from 50 to 200 Z1 L⁻¹. This would suggest that the larvae can be grown at 200 Z1 L⁻¹. Variation in the final survival between replicate tanks also seemed to decrease at higher densities. For *S. paramamosain*, Djanahid, Maridjo Wille, Kontara and Sorgeoos (2003) found a tendency of increased survival to 25 as a function of the Z1 stocking density (i.e. survival...
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Rates of 27%, 39% and 63% being obtained at densities of 50, 75 and 100 ZL−1 respectively. Baylon and Pailaman (1999) also reported higher survival and metamorphosis of Scylla serrata at 50 ZL−1 compared with lower densities of 30 and 25 ZL−1. Increased survival at higher larval densities somehow seems contradictory. However, indirectly, food ration might be responsible. Excess food in treatments with low larval densities may pollute the water and may thus cause mortality. In our study, we noted higher concentrations of ammonia and nitrite in the treatment having 50 ZL−1 (see Table 2). For the highest stocking densities tested in our study (200 ZL−1), the larval development rate seemed slightly impaired. This high stocking density may have caused competition for feed, resulting in slower development. Therefore, Z1 stocking densities in the range of 100–150 ZL−1 might be optimal.

Rotifer density for feeding early larval stages (Z1–Z2 stages)

Although there was a trend towards increased survival and growth with increasing rotifer density, no significant differences in larval survival or growth were found between the different rotifer densities tested. Although not significant, the highest survival was generally observed at 45 rotifers mL−1, while a density of 60 rotifers mL−1 resulted in the fastest larval development. The differences were, however, not very marked, and moreover such high feeding rates might be economically unrealistic. We can therefore conclude that feeding 30 rotifers mL−1 is enough for optimal larval performance. In practice, however, the intermediate density of 45 rotifers mL−1 was frequently used for feeding early larval stages. Other studies indicated that high rotifer densities (30–80 mL−1) are required for optimal growth and survival of S. paramamosain (Djunaibah, Madjopiono, Laveens & Wilee 1998; Zeng & Li 1999) and S. serrata (Suprayudi, Takouchi, Hamasaki & Hirokawa 2002). For S. paramamosain larvae, feeding 30 and 60 rotifers mL−1 resulted in a significantly higher survival compared with feeding only 15 rotifers mL−1 (Djunaibah et al. 2003). These authors found that the individual dry weight of Z5-fed 15 rotifers mL−1 was significantly lower than those of Z5 fed with higher rotifer densities. Practically, feeding 30 rotifers mL−1 at Z1 and increasing gradually to 45 mL−1 at Z2 proved to be sufficient for a stocking density of 100 ZL−1 in our trials in larger rearing tanks (500–1000 L). Increasing the ration by larval stages in this way compensates for the increased ingestion of crab larvae as they grow (Baylon, Bravo & Mami 2004). For early larvae, however, food amount cannot be reduced to their maximum ingestion potential as they are quite inefficient predators and therefore might require a minimal density to maximize encounter.

Similar to our study most studies investigating the effect of rotifer density added the live food in one single ration. Under these circumstances, the larval densities are only attained upon feeding and gradually decrease as larval consume the prey. Optimal live food quantities cannot, however, be separated from feeding frequency. Because zoea larvae can consume their optimal ration within 1 h, Genodepa, Southgate and Zeng (2004) suggested that they can be fed once a day. Because of the severe reduction in the nutritional value of rotifiers with longer retention times in rearing containers (Makridis & Olsen 1999) and the fact there is a minimum prey density needed for the passive feeding behaviour of zoea larvae (Heasman & Fielder 1983, Zeng & Li 1999), the interaction between the optimal ration and feeding frequency should be further investigated.

Artemia for feeding later larval stages (from Z3 onwards)

We found no difference between feeding Z3 a daily feed ration of 10, 15 or 20 Artemia nauplii mL−1. Especially in water larval stages (Z4–Z5), there was, however, a tendency towards higher survival with increasing ration. In this respect, it might be beneficial to increase the Artemia density by crab stage from 10 to 15 mL−1. High live feed densities would increase the chance for early larvae to encounter and capture food organisms (Zeng & Li 1999) and therefore would improve the larval performance (Brick 1974; Heasman & Fielder 1983; Quintero et al. 2001). On the other hand, older larval have a higher ingestion capacity. Optimal rations should therefore be determined for each larval stage separately. In this respect, studies on individual larvae are very useful to determine prey consumption. According to our previous experiments (Nghia 2004), each Z3, Z4, Z5 and megalopa stage was capable of consuming on average 15, 25, 37 and 114 newly hatched Artemia day−1 respectively. Therefore, at a stocking of 100 larvae L−1, the daily Artemia feeding densities theoretically should be at least 1.5, 2.5, 3.7 and 11.4 mL−1 for Z3, Z4, Z5 and megalopa stages respectively. For Z1, Z2 and Z3 stages of S. serrata, the
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The number of Artemia nauplii ingested by the larvae at a lower food density of 2.5 mL$^{-1}$ was comparable to that at 5 mL$^{-1}$, and for 24–25, at 5 mL$^{-1}$, it was comparable to 10 mL$^{-1}$ (Baylon et al. 2004). In that study, Artemia was, however, co-fed with rotifers at a density of 15–20 mL$^{-1}$. If Artemia was the only food, the optimal Artemia ration would therefore probably be higher than 2.5–5 mL$^{-1}$. In another study on S. serrata, a daily optimum food concentration of 10 Artemia nauplii mL$^{-1}$ was established for zoea survival (Brick 1974). In the mass seed production of S. serrata, newly hatched Artemia are given starting late 27 at 0.5–3 mL$^{-1}$ and 5–7-day-old Artemia are routine fed from late 25 to early megalopa (Ottnino & Parroño-Estapa 2003). Older Artemia provided a larger-sized prey for zoea to megalopa and hence, the density was reduced.

For megalopa of S. panamensis, we found a thousandfold higher number of ingested newly hatched Artemia nauplii compared to 25 (114 and 37 Artemia respectively) for a similar prey density (Nghia 2004). This means that megalopa are voracious predators, capable of chasing their prey actively and consume large amounts of feed in a short time. From this, it could be beneficial if megalopa are fed frequently smaller rations in order to optimize feed quality and reduce cannibalism. Gencedepo et al. (2004) similarly indicated that in contrast to earlier larval stages, which can be fed once per day, S. serrata megalopa may need to be fed more often to maximize ingestion. These authors found no significant differences in the ingestion rate of megalopa fed microbound diets at rates ranging from 12.5% to 100% of the standard ration (equivalent to 5 Artemia nauplii mL$^{-1}$ in 1 h). Baylon et al. (2004) also found a high increase in Artemia ingestion in the first few days of the planktonic phase of the megalopa stage. Later on, megalopa become more benthic as they prepare for the second metamorphosis to first crab. Swimming Artemia are no longer accessible and minced shrimp or mussel meal are a more suitable feed.

In conclusion, a ration of 10 Artemia nauplii mL$^{-1}$ appears to be sufficient for the optimal performance of z3 larvae. An increase in prey density in the 24–25 stage may, however, be beneficial. These food amounts appear to be higher than what most other studies recommend (2.5–10 Artemia nauplii mL$^{-1}$). Brick (1974); Baylon et al. (2004). Perhaps the recirculating system used in this study resulted in a greater loss of prey organisms (e.g. more Artemia were entombed on the overflow screen) than in the small batch culture systems used in other experiments. The small nauplii size of the Artemia strain (Vinh Chau strain) used in our study could be another reason that led to increased ingestion. In practice, (larger sized) highly unsaturated fatty acid-enriched Artemia were normally used in order to reduce the prey amount to 5–10 mL$^{-1}$. Megalopa probably should be fed more frequently and, towards the end of that developmental stage, a non-moving food may be better.

**Prophylactic chemicals**

Laboratory cultures of crab larvae often suffer severe mortality from disease, particularly from epibiotic bacteria and larval mycosis (Armstrong, Buchanan & Caldwell 1976; Harnasahi & Haiai 1993 a, b). A study on S. serrata indicated a significantly higher survival up to DAFT 7 (over 90%) when using oxytetracycline, whereas almost complete mortality occurred in the control treatment (Mann 2001). The author considered that potentially up to 80% of the larval mortality could be attributed to bacteriological causes. The results of our study also indicated that bacteria are, more than any other factor tested, a main cause of larval mortality. Antibiotics more than doubled survival up to the crab stage.

However, antibiotics have not always been used in a responsible manner in aquaculture. A major consequence of using antibiotics has been the proliferation of resistant bacteria and the transmission of resistance to other bacterial species (Benson 1998). The development of antibiotic resistance by pathogenic bacteria is considered to be one of the most serious risks to human health at the global level (FAO 2002). Formalin is more acceptable than antibiotics as it shows no accumulation in animal tissues (Jung, Kim, Jeon & Lee 2001). Recently, however, Japan has strictly banned the use of formalin in aquaculture as it may cause cancer in humans, reduces oxygen levels in the water and causes algae to die off (NASEP 2003). Moreover, in our experiments formalin did not significantly improve larval survival compared with the negative control. Pathogenic bacteria are considered to be one of the most serious causes for the high mortality of early crab larvae. It can be safely assumed that all inputs (seawater, broadstock, live feed and daily management in hatcheries) into the culture tank are potential sources of infection (Blackshaw 2001). Strict hygiene at all steps is always advised for hatchery activities. However, this advice is not always followed, especially in backyard hatcheries. Therefore, other techniques should be
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Investigated as alternatives for the use of chemicals. Ozoneation and probiotics could be interesting in this respect (Davis 2003; Nghia 2004). Ozone is a powerful oxidant and is becoming more and more popular in various aquaculture systems for disinfection and improving water quality by oxidation of inorganic and/or organic compounds (Tango & Gagnon 2003). In our study, direct application did not significantly improve survival compared with a negative control. However, there was a tendency of a residual O3 concentration of 0.06 mg L⁻¹ to improve larval performance. Variability within this treatment was, however, very high, which could be indicative of the fact that dosing was not careful enough. Longer O3 exposure times (4–10-min exposure, equivalent to 0.12–0.19 mg L⁻¹ residual O₃) all decreased overall survival. These high O3 concentrations probably caused physical damage to the crab larvae. In Penaeus monodon juveniles, 0.34–0.5 mg L⁻¹ residual O₃ caused loss of balance, immobility and destruction of the gill lamellar epithelium (Manopop, Lopinyosiri & Menasvat 2003). Ozone treatment should therefore be investigated further, with determination of proper doses for each larval stage. Ultimately, the microbial flora will need to be controlled and there is evidence that this can be achieved using recirculating systems in which O₃ treatment is combined with the inoculation of the biological filter with selected nitrifying and probiotic bacteria (Gatesoupe 1991; Rombut, Nanwika, Bonn, Mierrens, Dhert, Top, Sorgeloos & Verstraete 2001).

Conclusions and suggestions

The combination of a green-water batch system for early stages and a recirculating system with microalgae supplementation for later stages, a stocking density of 100–150 21⁻¹, a feeding density of 30–45 rotifers ml⁻¹ for early stages and 10–15 Artemia nauplii ml⁻¹ for later stages are recommended for larval rearing of S. paramamosain.

The optimal ration for crab larvae should, however, be adjusted depending on various factors, e.g. species, larval stages, larval status, gray size, rearing system and rearing techniques. A feeding regime with frequent addition of small quantities of feed is worth investigating.

Antibiotics improved larval survival, proving again that bacterial interference is one of the major causes of mortality. Formalin could not significantly improve survival compared with the control. Both products, moreover, are not encouraged for commercial mud crab larviculture as they are unsafe. Direct ozonation as an alternative to prophylactic chemicals is worth investigating.

Acknowledgments

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