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The effect of poly β-hydroxybutyrate on larviculture of the giant freshwater prawn *Macrobrachium rosenbergii*

Dinh The Nhan [a,b], Mathieu Wille [a], Peter De Schryver [c], Tom Defoirdt [a], Peter Bossier [a], Patrick Sorgeloos [a,*]

[a] Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Rozier 44, 9000 Gent, Belgium
[b] Faculty of Fisheries, Nong Lam University, HCM City, Vietnam
[c] Laboratory of Microbial Ecology and Technology, Ghent University, Coupure Links 653, 9000 Gent, Belgium

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ABSTRACT

In this study, we investigated the effect of poly β-hydroxybutyrate (PHB) on the culture performance of larvae of the giant freshwater prawn *Macrobrachium rosenbergii* and on the bacterial levels inside the larval gut. Instar II *Artemia* nauplii were cultured with or without PHB (5 g l^{-1}) and/or a lipid emulsion rich in highly unsaturated fatty acids (HUFA) for 24 h. The effect of feeding PHB and/or HUFA-enriched *Artemia* nauplii on the performance of *Macrobrachium* larvae was investigated. Feeding larvae of the giant freshwater prawn with PHB-containing *Artemia* nauplii significantly increased survival and development of the larvae. Moreover, total bacterial counts and *Vibrio* spp. counts were found to be significantly lower in PHB-fed larvae when compared to control larvae, indicating that the PHB addition had a growth-inhibitory effect towards these potentially pathogenic microorganisms. Finally, a combination of PHB addition and lipid enrichment resulted in the best overall culture performance since it significantly improved larval survival as well as larval development. The optimal PHB concentration and formulation for bio-encapsulation into *Artemia* should be investigated further to increase the economical efficiency of *Macrobrachium* larval production.

1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879), is a commercially important aquaculture species. Its culture has expanded rapidly not only within Asia but also in regions far remote from the natural distribution of the species (FAO, 2000). However, availability of healthy and high-quality seeds has always been a major obstacle in the expansion of *M. rosenbergii* farming. One of the major factors that is hampering the quality of hatchery-reared seed, culminating in low yield because of mass mortality, is disease. Outbreaks of disease in prawns are often attributed to bacterial infections (Sung et al., 2000; Phatarpekar et al., 2002; Al-Harbi, 2003). Mass mortality of larvae in the hatcheries restricts the regular production of high-quality post-larvae. Such mortality is often attributed to opportunistic pathogenic bacteria (Skjermo and Vadstein, 1999). *Vibrios* have been reported as the causative agents for numerous disease outbreaks (Alavandi et al., 2004; Kennedy et al., 2006) and are often reported as a major problem in prawn hatcheries (Nayak and Mukherjee, 1997; Jayaprakash et al., 2006). Antibiotics and disinfectants, the conventional approaches to control bacterial populations in prawn hatcheries, are quite popular. However, they have begun to be withdrawn from the market in many countries owing to concerns about public health and environmental safety (Schneider et al., 2003). Moreover, the use of low doses of antibiotics as prophylactics has resulted in the development of antibiotic resistance (Teo et al., 2000, 2002), rendering antibiotic treatments ineffective in controlling diseases (Karunasagar et al., 1994). Therefore, there is an urgent need to search for eco-friendly alternative control techniques which help to maintain the animals' health.

Considering that antibiotics should no longer be used as animal growth promoters, there is currently a significant interest in short-chain fatty acids (SCFAs) as biocontrol agents in animal production (Defoirdt et al., 2006). Several studies have shown that SCFAs inhibit the growth of yeasts and enterobacteria such as *Salmonella typhimurium, Escherichia coli* and *Shigella flexneri* (Cherrington et al., 1991; Bearson et al., 1997; Sun et al., 1998; Van Immerseel et al., 2003). SCFAs have previously been shown to inhibit or decrease the growth of *Salmonella* in chickens (Waldroup et al., 1995; Van Der Wielen et al., 2000; Van Immerseel et al., 2005) and of pathogenic luminescent *Vibrios* in *in vitro* tests (Defoirdt et al., 2006). Moreover, SCFAs were shown to significantly increase the survival of challenged brine shrimp (*Artemia*) nauplii. In addition, SCFAs might also provide with energy for shrimp (Defoirdt et al., 2006). Effective fatty acid concentrations are rather high and consequently, it would not be economically feasible to dose fatty acids in the culture water of an aquaculture system in order to protect the animals. Moreover, the addition of high levels of organic carbon in the water would give rise to an excessive growth of heterotrophic bacteria which might have a

* Corresponding author. Tel.: +32 926 43753; fax: +32 92644193.
E-mail address: Patrick.Sorgeloos@UGent.be (P. Sorgeloos).

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negative effect on the health of the animals (because of oxygen depletion and/or because the growing bacteria could be pathogenic). Polyhydroxyalkanoates (PHAs) are water-insoluble polymers of β-hydroxy short-chain fatty acids that are produced as a reserve material by numerous bacteria (Anderson and Dawes, 1990). Interestingly, different studies provided some evidence that PHAs can also be degraded upon passage through the gastrointestinal tract of animals and consequently, adding these compounds to the feed might result in biocontrol effects similar to those described for SCFAs (Defoirdt et al., 2009). Defoirdt et al. (2007) reported that the SCFA β-hydroxybutyrate was able to inhibit the growth of a virulent *Vibrio campbellii* strain *in vitro*. Based on this result, the authors investigated whether the polymer of this SCFA, the well-known bacterial storage compound poly-β-hydroxybutyrate (PHB), could be used to protect aquatic animals from pathogenic bacteria. The addition of commercial PHB particles (average diameter of 30 μm) at a concentration of 1000 mg l^{-1} to the culture water resulted in a complete protection of brine shrimp from the pathogenic *V. campbellii* (Defoirdt et al., 2007).

In this study, we investigated the effect of PHB (administered via the live food) on the survival and growth of larvae of the giant freshwater prawn *M. rosenbergii* and on the microbiota (total bacteria and *Vibrio* spp.) associated with the larvae. To demonstrate the application potential, loading of the *Artemia* nauplii with PHB was combined with lipid enrichment, a technique which is routinely applied in many fish and crustacean hatcheries.

2. Materials and methods

2.1. Experimental animals

The study included 2 experiments on *M. rosenbergii* larval rearing performed in 1–l glass bottles. Adult *M. rosenbergii* imported from Thailand were used as brooders. The water quality parameters of the broodstock tanks, the photoperiod, and the feeding regime were adjusted in accordance to the recommendations for prawn rearing (New, 2003). Water was exchanged at a rate of approximately 20% per day after removing waste and uneaten feed by siphoning. NH_4–N; NO_2–N, and NO_3–N levels were maintained below 0.2, 0.1, and 10.0 mg l^{-1} respectively. The photoperiod was set at 12 h light at an intensity of 600 lx with fluorescent lamps at the water surface. Temperature was maintained at 28 ± 1 °C. Prawns were fed *ad libitum* with a commercial formulated shrimp diet twice a day (at 9:00 h and 17:00 h). Larvae were obtained from a single ovigerous female breeder (Cavalli et al., 1999, 2000; Baruah et al., 2009). The newly-hatched larvae were grown for 4–10 days before being stocked into the experiments. Twenty-four hours after hatching, the larvae were transferred into 10-l cylindro-conical jars connected to a single recirculation system as was described by Cavalli et al. (2001). Water was exchanged at a rate of approximately 50% per day after removing wastes and uneaten feed by siphoning. NH_4–N; NO_2–N, and NO_3–N levels were maintained below 0.2, 0.1, and 10.0 mg l^{-1} respectively. Water salinity was adjusted by diluting seawater to 12 g l^{-1} with deionized water. Temperature was maintained at 28 ± 1 °C. Gentle aeration was applied in all rearing jars. A fluorescent lamp system was installed, providing around 900–1000 lx at the water surface for 12 h day^{-1}. Newly-hatched *Artemia franciscana* nauplii (EG® type, Batch 041004, INVE Aquaculture, Baasrode, Belgium) were used as live food and administered via the *Macrobrachium* culture water *ad libitum* (density always over 6 *Artemia* nauplii ml^{-1}) from day 2 to day 10. The *Artemia* dosage was split over two feedings at 9.00 h and 17.00 h.

2.2. Experimental design

The experiments were performed in glass cones containing 1000 ml brackish water (12 g l^{-1} salinity). The glass cones were placed in an aquarium tank containing water maintained at 29 ± 1 °C using a thermostatic heater. A lamp system was installed to provide around 900–1000 lx at the water surface for 12 h day^{-1}. The cones were supplied with gentle aeration to ensure dissolved oxygen in the rearing water was always over 5 mg l^{-1}. In all experiments, an open clear water system was used with a daily water exchange of 50%. During water exchange, the remaining *Artemia* and waste from the previous day were removed by siphoning. This operation was carried out with great care to avoid loss of larvae. Feeding was done after water exchange. The larvae were exclusively fed *A. franciscana* (Great Salt Lake strain) nauplii *ad libitum* twice a day at 9:00 and 17:00 during the complete experimental period. Depending on the treatment, instar II *Artemia* nauplii were enriched with PHB particles (average diameter 30 μm, Lot: S68924-488, Sigma-Aldrich, Bornem, Belgium) at a concentration of 5 g l^{-1} *Artemia* culture and/or a lipid emulsion (ICES 30/0.6/C, containing 30 % total n−3 HUFA with a DHA/EPA ratio of 0.6, Lot: 903003.01, Han et al., 2000) at a concentration of 0.6 g l^{-1} *Artemia* culture water during 24 h before feeding them to the *Macrobrachium* larvae. In the control treatment, *Artemia* nauplii were treated in the same way, without enrichment of either PHB or the lipid emulsion.

Experiment 1 consisted of two treatments of feeding the *Macrobrachium* larvae with PHB enriched *Artemia* nauplii or control nauplii (without enrichment). At the start of the experiment, 10 days-old larvae were stocked at an initial density of 50 l^{-1}. Experiment 2 consisted of 4 treatments with the addition of PHB and/or a lipid emulsion and the control without any enrichment (+P+L: PHB and lipid added; +P−L: PHB added only; −P+L: Lipie added only; −P−L: nothing added). In this experiment, 4 day-old larvae were stocked at an initial density of 100 l^{-1}. The experimental duration was 15 days for Experiment 1, and 28 days for Experiment 2. In both experiments, the treatments were performed in six replicates.

2.3. Analyses

2.3.1. Detection of PHB in Artemia nauplii used to feed Macrobrachium

Instar II *Artemia* nauplii were enriched with PHB particles (average diameter 30 μm, Lot: S68924-488, Sigma-Aldrich, Bornem, Belgium), which were added to the culture water at a concentration of 5 g l^{-1}. PHB was detected in the nauplii following the methodology described in Defoirdt et al. (2007). Briefly, after 15 min of incubation, 10 nauplii were killed with absolute ethanol and stained with the fluorescent dye Nile Blue A (Ostle and Holt, 1982). The nauplii were examined with an Axioskop II microscope (Carl Zeiss, Jena, Germany) equipped with a Peltier-cooled single-chip digital camera (Orca Illm; Hamamatsu, Massay, France) connected to a PC.

2.3.2. Larval stage index

At days 10 and 15, larval development in each treatment was estimated through determining the larval stage index (LSI) according to Maddox and Manzi (1976). The average larval stage of 60 larvae from each treatment was recorded based on the description by Uno and Kwon (1969).

2.3.3. Larval survival

Larval survival was checked at days 5, 10 and 15 in Experiment 1. In Experiment 2, larval survival was checked at days 10, 15, 20 and 28. Counting the larvae was carried out with great care to avoid stress of the larvae.

2.3.4. Bacteria in the larval gut

Prawn larvae samples were collected at the beginning and on the last day of each experiment. Three samples of ten larvae were taken randomly from all replicates for analysis of bacterial counts in the prawn gut. Surface bacteria were removed according to a procedure described by Huys et al. (2001). The prawn sample was first immersed in a benzocaine solution (Sigma, 0.1%) for 10 s, transferred to a benzalkonium chloride solution (Sigma, 0.1%) for another 10 s, and

rinsed three times with autoclaved nine salts solution (NSS) (Olsson et al., 1992) for 5 s each time. The larvae were then transferred to a sterile plastic bag containing 10 ml of NSS, and homogenized with a stomacher blender for 6 min. The water samples and the prawn homogenates were serially diluted in NSS. Fifty µl from each dilution was plated on Marine Agar, and on Thiosulphate–Citrate–Bile Salt–sucrose (Biokar Diagnostics, France) for enumerating the total culturable bacteria, and *Vibrio* spp., respectively. The number of colonies was counted after incubation at 28 °C for 48 h.

2.3.5. Statistics

Larval stage index, larval survival and countable bacterial density were analyzed by analysis of variance (one-way ANOVA) and, if significant differences were found ($P<0.05$), the least significant differences (Duncan, SPSS version 13.0 software) test was applied. All percentage data were normalized by arcsine transformation for statistical analysis, but only non-transformed means are presented.

3. Results

3.1. Experiment 1

In a first experiment, the effect of feeding PHB to *Macrobrachium* larvae by enriching it into the live food was studied. Instar II *Artemia* nauplii used as live food for the *Macrobrachium* larvae were cultured with and without PHB (5 g l^{-1}). Microscopic analysis showed that the gut of the *Artemia* nauplii in the treatment without PHB was empty (Fig. 1), whereas in the treatment with PHB, the upper part of the gut was almost completely filled. Epifluorescence images of Nile Blue A stained nauplii showed that the gut content of PHB-treated nauplii was brightly fluorescent and could clearly be distinguished from the (auto) fluorescence of the nauplii, which indicates that the nauplii had ingested the PHB particles. The effect of adding PHB to the live food on the performance of *Macrobrachium* larvae was studied by determining the larval survival and development over a culture period of 15 days. Evaluation of the larval development based on the larval stage index showed that larvae in the PHB treatment had grown significantly better than those in the control treatment (no PHB addition) after 10 days ($P<0.05$) (Fig. 2). Larval survival was determined at days 5, 10 and 15 (end of experiment) and was always significantly higher in the PHB treatment than in the control treatment ($P<0.05$). The difference in survival became more and more pronounced towards the end of the experiment (Fig. 3).

3.2. Experiment 2

In this experiment, the effect of a combination of PHB and a lipid emulsion (both administered through the live food) on the performance of *Macrobrachium* larvae was investigated. The treatments with lipid enrichment showed the highest value of LSI, while the control treatment had the lowest larval development. The LSI in the treatment with PHB was significantly higher than in the control treatment, but it was lower than in the treatments with lipid enrichment (Fig. 4). There was a significant interaction of feeding PHB and lipid enrichment with respect to larval stage index at days 10 and 15 ($P<0.05$). The larval survival was checked at four different time intervals during the experiment. The differences between the treatments were similar at all time points. The treatment with both PHB and lipid enrichment resulted in the highest survival (56% at the end of the experiment), while the survival in the control treatment was the lowest (12% at the end of the experiment). The treatments with either PHB or lipid enrichment alone were intermediate and were not significantly different from each other (Fig. 5). After 28 days of the experiment (32 days after hatching), 90% of the larvae in the treatments with lipid enrichment had reached the postlarval stage, while in the treatment with PHB only around 50% had metamorphosed, and in the control treatment only 10% of the animals were postlarvae. There was no interaction between PHB and lipid enrichment with respect to larval survival.

Total bacteria and *Vibrio* levels in the prawn larval gut were determined at the beginning and at the end of the experiment by plate

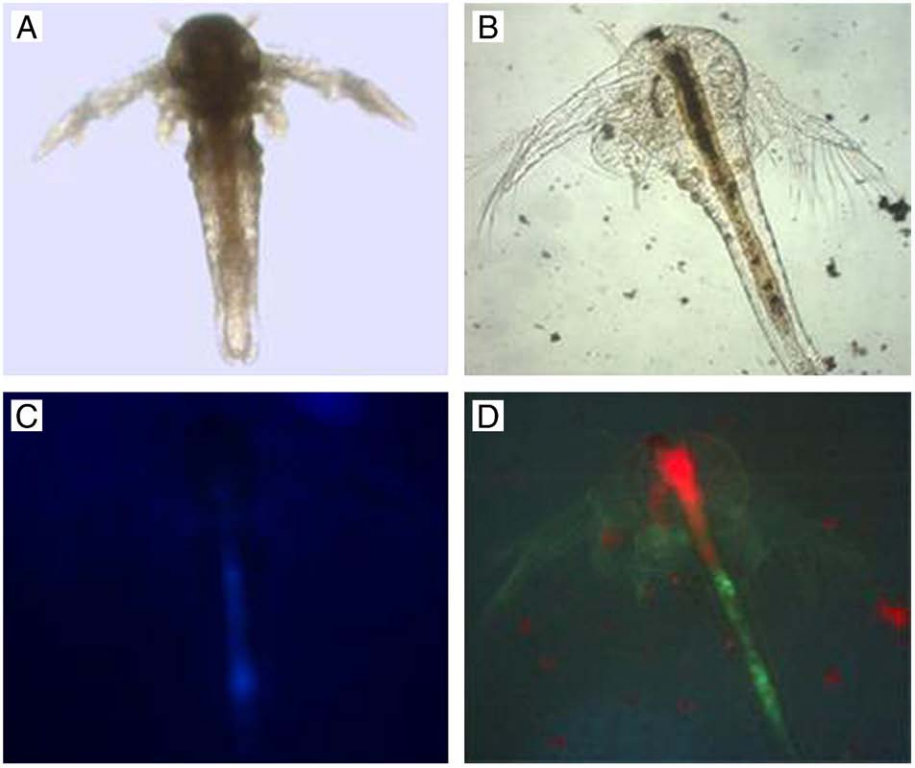

Fig. 1. Representative light (upper row) and epifluorescence microscopy (lower row) images of Nile Blue A stained *Artemia* nauplii after 15 min without (panels A and C) and with (panels B and D) PHB added to culture water at 5 g l^{-1}.

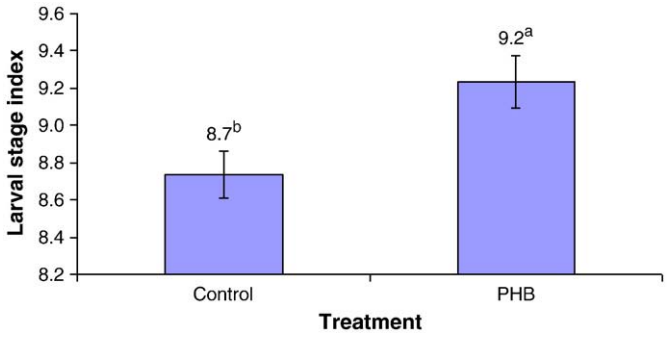

Fig. 2. Larval stage index at day 10 of the test (20 days after hatching) of *Macrobrachium rosenbergii* larvae fed *Artemia* nauplii with or without PHB enrichment in experiment 1. Values are average ± SE, $n = 6$. Different superscript letters denote significant differences ($P<0.05$).

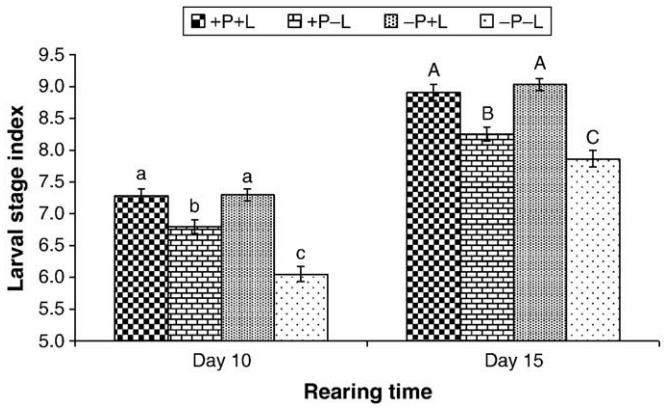

Fig. 4. Larval stage index at days 10 and 15 of *Macrobrachium rosenbergii* larvae fed *Artemia* nauplii with or without PHB and/or lipid enrichment in experiment 2. +P+L: PHB and lipid added; +P−L: PHB added only; −P+L: Lipie added only; −P−L: Nothing added. Values are average ± SE, $n = 6$. Different letters denote significant differences ($P<0.05$). Small and capital letters denote different comparisons.

counting on Marine Agar and TCBS Agar, respectively. At the end of the experiment, the total bacterial number in the larval gut was significantly higher in the treatment with lipid enrichment only (reaching up to 28×10^4 CFU larva^{-1}) when compared to the other treatments. The *Vibrio* numbers in the larval gut at the end of the experiment were significantly higher in the treatments without PHB when compared to the treatments with PHB addition. The number of *Vibrio* spp. in the larval gut was initially 21 ± 6 CFU larva^{-1}, and increased to $1.3–2.2\times10^3$ CFU larva^{-1} at the end of the experiment in the treatments without PHB (Table 1). There was a significant interaction between feeding with PHB and lipid emulsion with respect to the microflora population density inside the larval gut ($P<0.05$).

4. Discussion

In this study, we investigated the effect of PHB on the culture performance of larvae of the giant freshwater prawn *M. rosenbergii* and on the bacterial levels inside the larval gut. *Artemia* nauplii are commonly used as live feed for *Macrobrachium* larvae (Lavens et al., 2000) and because Defoirdt et al. (2007) reported before that PHB can be accumulated by *Artemia* when added to the culture water, we delivered PHB to the prawn larvae via *Artemia*. The accumulation of PHB particles in the *Artemia* gut was confirmed by using fluorescent microscopy (Fig. 1). It was not possible to detect PHB in the gut of the *Macrobrachium* larvae because the larvae were not sufficiently transparent for fluorescence microscopy and may be because PHB content was much too small to allow detection by chromatography. The results presented in this paper showed that feeding *Macrobrachium* larvae with PHB-containing *Artemia* nauplii significantly improved larval survival (Figs. 3, 5). In Experiment 2 where the combination of PHB and a lipid emulsion rich in highly unsaturated fatty acids was studied, the highest larval survival was noted in the treatment with addition of both PHB and the emulsion (Figs. 3, 5). The treatments with PHB only and lipid emulsion only also significantly increased the survival of the larvae, though less pronounced than in the treatment with both additives. These results are in accordance to the report of Defoirdt et al. (2007), who found that PHB significantly increased the survival of brine shrimp larvae challenged with pathogenic *Vibrio* spp. The low survival of prawn larvae in the control treatment in this study was probably due to the presence of opportunistic bacteria that are associated with the larvae (as also reported by Baruah et al., 2009). In our study, we found that feeding *Macrobrachium* larvae with PHB-containing *Artemia* nauplii resulted in significantly lower levels of total bacteria and *Vibrios* (Table 1), which are frequently associated with diseases of aquatic organisms (Lightner, 1996; Otta et al., 2001). Phatarpekar et al. (2002) investigated the bacterial flora on prawn larvae and found that *Vibrio* spp. were detected in eggs and water but were conspicuously absent in larvae. However, the total viable count in larvae varied from 2.5×10^4 to 1.6×10^8 CFU g^{-1}. In the current study, total bacteria and total *Vibrio* counts in larvae of treatments without PHB addition varied from 15.9 to 28.0×10^4 and 0.4 to 22.0×10^2 CFU larva^{-1},

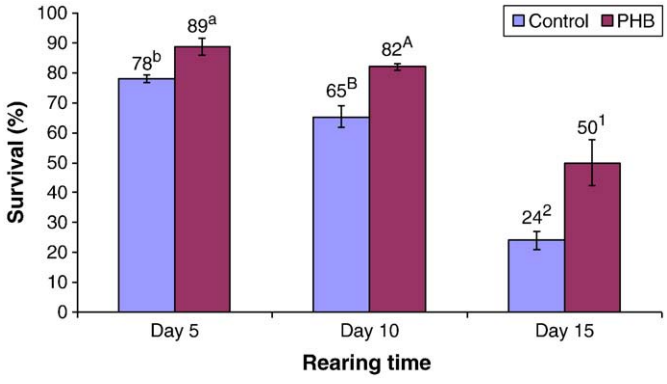

Fig. 3. Larval survival of *Macrobrachium rosenbergii* larvae fed *Artemia* nauplii with or without PHB enrichment after 5, 10 and 15 days of testing in experiment 1. Values are average ± SE, $n = 6$. Different letters denote significant differences ($P<0.05$). Different types of superscript denote different comparisons.

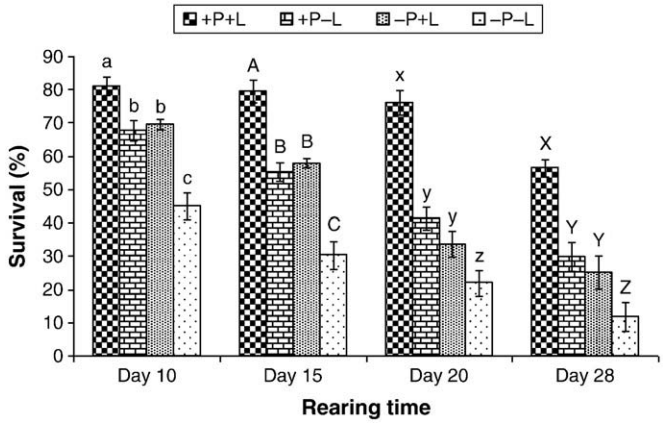

Fig. 5. Larval survival of *Macrobrachium rosenbergii* larvae fed *Artemia* nauplii with or without PHB and/or enrichment in experiment 2. +P+L: PHB and lipid added; +P−L: PHB added only; −P+L: Lipie added only; −P−L: Nothing added. Values are average ± SE, $n = 6$. Different letters denote significant differences ($P<0.05$). Different types of letters denote different comparisons.

Table 1
Microflora in the gut of *Macrobrachium rosenbergii* larvae fed *Artemia* nauplii with or without PHB and/or lipid enrichment in Experiment 2.

Treatment	Total bacteria (10^4 larva^{-1})		Total *Vibrio* (10^2 larva^{-1})	
	Initial	Final	Initial	Final
+P+L	3.2±0.2	5.6±1.1[a]	0.2±0.1	0.3±0.1[a]
+P−L		5.4±0.8[a]		1.6±0.7[a]
−P+L		28.0±2.5[b]		22.0±5.3[b]
−P−L		6.8±1.0[a]		13.3±1.8[b]
Interaction between PHB and nutrient emulsion		$P=0.002$		$P=0.008$

Values are average±SE, $n=3$. Different letters within column denote significant differences ($P<0.05$).

respectively, while in the PHB treatments they were only 1.7 to 5.6×10^4 and 0.3 to 1.6×10^2 CFU larva^{-1}, respectively.

Defoirdt et al. (2007) found that PHB particles protected brine shrimp larvae from pathogenic *V. campbellii* and hypothesised that PHB particles were (partially) degraded into β-hydroxybutyrate in the shrimp gut and that the release of this fatty acid protected the shrimp from the pathogen in two ways, i.e. by providing the shrimp with energy (resulting in a gut epithelium that is more resistant to infection) and by inhibiting the growth of the pathogen. This can also explain why feeding the *Macrobrachium* larvae with PHB-containing *Artemia* nauplii did increase survival and growth of the larvae when compared with the control. However, administration of a lipid emulsion significantly increased development of the larvae when compared with addition of only PHB. Indeed, the larvae could have used breakdown products of the PHB particles as an energy source, but they may have lacked essential nutrients required for growth, which were present in the lipid emulsion. Lipids are known to play several essential roles in crustacean larval development (Teshima 1972, 1997; Middleditch et al., 1980; Teshima and Kanazawa, 1983; Harrison, 1990). Apart from being a major source of metabolic energy and the main form of energy storage, lipids also supply essential fatty acids needed for the maintenance and integrity of cellular membranes, and serve as precursors of steroid and moulting hormones (Teshima, 1972; Harrison, 1990). Fatty acids released from PHB are short-chain fatty acids, that may provide an extra energy source, but do not provide long chain fatty acids necessary for optimal metabolic functioning. Several studies investigated the lipid metabolism of larval and juvenile stages of the freshwater prawn (Devresse et al., 1990; Sheen and D'Abramo, 1991; Teshima et al., 1992, 1997; D'Abramo and Sheen, 1993; Querijero et al., 1997; Roustaian et al., 1999). Feeding the larvae with a lipid emulsion rich in highly unsaturated fatty acids (HUFA) both provided the larvae with an excellent source of energy (Tidwell et al., 1998), and the necessary structural components for tissue growth and hormone synthesis, resulting in better growth. This result was in accordance with several previous studies, for example Romdhane et al. (1995) that demonstrated that the longer the period of feeding ($n-3$) HUFA-enriched *Artemia* nauplii, the better the results in terms of growth, metamorphosis rate, survival and stress resistance of prawn larvae. Similarly, Sorgeloos and Léger (1992) and Alam et al. (1995) reported that the application of live food supplementation with marine oils rich in n−3 fatty acids improved the larval growth of *M. rosenbergii*.

Degradation of PHB can occur via several mechanisms, including chemical decomposition or hydrolysis and enzymatic hydrolysis (Defoirdt et al., 2009). However, the exact mechanism by which the PHB polymers are broken down inside the intestinal tract of animals, i.e. whether it is mainly driven by physico-chemical properties of the gut environment or by the release of digestive enzymes by the host and/or by microorganisms present in the gut, is not yet known. Defoirdt et al. (2007) reported that PHB-degradation in brine shrimp larvae was most probably physico-chemical or mediated by enzyme activity of the brine shrimp and not microbial since the larvae were axenic. The *Macrobrachium* larvae used in this study, in contrast, were not axenic and consequently, the microorganisms associated with the larvae might also have contributed to PHB breakdown. Indeed, enzymatic degradation of PHB by PHB-depolymerase producing microorganisms is well-documented (Doi et al., 1990; Yoshie et al., 1999; Quinteros et al., 1999; Jendrossek and Handrick, 2002; Choi et al., 2004; Khanna and Srivastava, 2004), although as far as we know no intestinal PHB-degrading microorganisms have been reported yet.

The application of PHB in aquaculture larval rearing, or more specifically in prawn larval production, may be constrained due to the current high cost of commercial PHB products. However, PHB can be produced relatively easily by *Bacillus* and *Lactobacillus* spp. (Anderson and Dawes, 1990; Aslim et al., 1998; Yilmaz et al., 2005) from inexpensive substrates, such as molasses, making this technique cost-effective and sustainable (Kim, 2000). Furthermore, Defoirdt et al. (2007) believe that it should be possible to produce PHB *in situ* in the culture water by adding C-rich compounds or by increasing the C/N ratio of the feed. All these concepts may open new opportunities to produce PHB with lower cost price in the future, with possible application in aquaculture and aquatic seed production.

In conclusion, the results obtained in this study showed that feeding larvae of the giant freshwater prawn *M. rosenbergii* with PHB-containing *Artemia* nauplii significantly increased larval survival and development. Moreover, total bacterial counts and *Vibrio* counts were found to be significantly lower in PHB-fed larvae when compared to control larvae, indicating that the PHB addition had a growth-inhibitory effect towards these potentially pathogenic microorganisms. Finally, a combination of PHB addition and a lipid emulsion resulted in the best overall culture performance since it significantly improved larval survival as well as larval development. The optimal PHB concentration and formulation for encapsulation into *Artemia* should be investigated further to increase the economical efficiency of the larval production.

Acknowledgments

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