Aquaculture Research

Aquaculture Research, 2009, **40**, 1428–1438 doi: 10.1111/j.1365-2109.2009.02241.x

Growth performance, survival and maturation of *Litopenaeus vannamei* (Boone) in an inland CRS with no water reposition

Abundio González-González[1], Roberto Mendoza-Alfaro[1], Gabriel Aguirre-Guzman[2] & Jesus Genaro Sánchez-Martínez[2]

[1]Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León. Av. Universidad s/n, Ciudad Universitaria. San Nicolás de los Garza, Nuevo León, México

[2]Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma de Tamaulipas. Km 5 Carr. Victoria – Mante, Cd. Victoria, Tam. México

Correspondence: G Aguirre-Guzman, Km 5 Carr. Victoria – Mante, Cd. Victoria, Tamaulipas, México, CP 87000. E-mail: gabaguirre@uat.edu.mx

Abstract

Closed recirculation systems (CRS) present an alternative for providing organisms to the aquaculture. A CRS with zero water exchange was used in the present study; the CRS consisted of a culture and maturation facility, biofilter system and reservoirs tanks. During two consecutive trials, the CRS efficiency was evaluated by assessing the growth, survival and maturation of juveniles into adults *Litopenaeus vannamei* (Boone). Throughout the study, water quality parameters (temperature, pH, salinity, NH_3, NO_2 and NO_3) and ion concentration were monitored. Most parameters showed fluctuations without significant differences. However, a decrease in pH was observed during the maturation phase, and an increase in phosphorus was detected, in both the trials, compared with that in initial seawater. Growth and survival for juvenile and pre-adult shrimps presented similar variations without significant differences. Female gonadal maturation and spawning rate were not significantly different between trials. Unfortunately, shrimp eggs underwent lysis 6 h after spawning. These results show that the growth, survival and maturation obtained under CRS conditions are reproducible, and suggest the possibility of using these systems for biosecure shrimp culture, protected against eventual diseases outbreaks. The results of this study also suggest the importance of future studies addressing ion concentration changes in a CRS with zero water exchange.

Keywords: closed recirculation system, growth, juvenile, maturation, shrimp

Introduction

The increase in human consumption of fish and shellfish has been partly compensated by aquaculture and secondary fisheries (FAO 2004). At present, cultured aquatic organisms have become the world's main seafood supplier (Farzanfar 2006). However, this industry relies mostly on open culture systems that have a negative environmental impact (Samocha, Lopez, Jones, Jackson & Lawrence 2004), and could facilitate the spread of disease. Disease alone is the cause of losses in several billion dollars (USD) by shrimp farms (Lightner 2003), a situation that may worsen, given its potential danger for disease dissemination in wild shrimp populations (Fegan 2001).

New techniques in aquaculture have focused on increasing production and solving disease and environmental problems. Physical filters, microbial biofilters and ultra violet (UV)-light treatment in closed recirculation systems (CRS) represent an alternative for the removal of solid waste, nitrogen products and undesired biota (Cytryn, Gelfand, Barak, Rijn & Minz 2003; Sharrer, Summerfelt, Bullock, Gleason & Taeuber 2005; Decamp, Conquest, Cody, Forster & Tacon 2007). Closed recirculation systems may prevent pathogen transmission, decrease culture water volume and avoid the discharge of contaminated

(organic matter and chemical products) water into the environment (Kuhn, Boardman, Craig, Flick & McLean 2007). Closed recirculation systems functioning on a zero water discharge basis (Cytryn *et al.* 2003; Decamp *et al.* 2007; Gelfand, Barak, Even-Chen, Cytryn, Rijn, Krom & Neori 2007), compared with conventional aquaculture systems, represent a viable culture alternative (Xiongfei, Zhidong, Deshang, Kangmei, Zhuanshang, Liegang, Kaichong & Bailin 2005) on an experimental and a commercial scale (Kaiser, Paulet, & Endemann 1998; Delabbio, Murphy, Johnson & Hallerman 2003; Peixoto, Cavalli & Wasielesky 2005; Tomoda, Fushimi, & Kurokura 2005).

Closed recirculation systems efficiency requires water treatment such as sedimentation, aeration and filtration, while also requiring the establishment of biosecure and controlled areas to lower the risks of disease and pollutants' introduction (Kaiser *et al.* 1998; Gandy, Samocha, Masser, Fox, Ali, Gatlin & Speed 2007). These measures are essential for the development of a sustainable, high-quality, specific pathogen-free (SPF) shrimp culture; however, the use of this technology introduces a new variable in production costs (Xiongfei *et al.* 2005).

The present study aimed to assess shrimp growth development, maturation and water-quality parameters in an experimental zero water exchange CRS, simulating the conditions of commercial production facilities.

Materials and methods

CRS

The CRS used in this study was designed to work with no water reposition. The facility where the CRS was established consisted of four sections: (I) biofilters and reservoir tanks; (II) culture room; (III) maturation room; and (IV) spawning room (Fig. 1).

Biofilters

Each culture room tank was fitted with an in-house-made individual biofilter system (IBS) consisting of two parts, (1) a submerged inverse biofilter, constructed with a 10 × 33 cm, D/L PCV cylindrical container with 0.6-cm diameter holes drilled at the bottom, filled with broken oyster shells (1200 cm^3, < 3 cm^2-sized particles) through which water was pumped up from the tank's bottom with an air lift system; (2) an individual external biofilter device made of PCV filled with synthetic fibre (40 μ, 200 cm^3, removed every 24 h), a polypropylene mesh net (200 cm^3, as a bacterial fixation substrate), and activated carbon–zeolite (200 cm^3, as a clarifying medium) where ammonia transformation into nitrate and nitrite took place (Kamstra, Van der Heul & Nijhof 1998; Lobo, Revah & Viveros 1999; Cytryn *et al.* 2003). Filtered water was then passed through UV light and returned to the tank by gravity. The water flow for the IBS was set at 1.5 L min^{-1} 24 h day^{-1} for each tank (288% of water exchange).

Each maturation room tank was fitted with an IBS and an alternative submerged inverse biofilter (ASIB) made with a 15 × 95 cm D/L PVC with 0.6-cm diameter holes drilled at the bottom filled with broken oyster shells (4000 cm^3, particle size of $\leq$ 6 cm^2). The ASIB was placed on the tank's central drain with a water level set at 70 cm. The effluent at the bottom of each tank was conducted to a bed of crushed oyster shells (6000 cm^3, particles size of $\leq$ 3 cm^2) as a wet-air biofilter, sock-filtered at 250 μ and then sent to a sediment tank (above-ground reservoir) by gravity (Gandy *et al.* 2007). The in-house-made external general biofilter (EGBF) was filled with 0.8-m^3 polypropylene netting used as a bacterial fixation substrate. The biofiltered water was stored in the above-ground reservoir and then sent to the maturation or spawning rooms by gravity, through a cartridge filter (10 μ) and eight (30-W) UV lights (Copeland, Watanabe & Dumas 2005). The daily water recycling flow for the ASIB was set at 2.5 L min^{-1} 24 h (262% of water exchange).

Water source and quality

Twenty-four cubic metres of sand-filtered seawater (32 g L^{-1}) from the Gulf of Mexico (La Pesca, Soto La Marina, Tam., Mexico) were transported 145 km inland, to the research facilities at the Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Tamaulipas (UAT), to be used in the CRS. Before its use in the system, the water was filtered through a 35-μ Jacuzzi™ (Magnum L.R., R.C. & Cygnet Pump, Toronto, ON, Canada) sand filter, with decreasing filter cartridges (20, 10 and 5 μ) and exposed to 12 UV lights (30 W) for 24 h (Copeland *et al.* 2005).

Brackish water (15 g L^{-1}) was made by mixing sea and freshwater from a deep well in order to fill six concrete culture-room tanks (1.2 m^3 each). Brackish water was stored in two 5.1-m^3 rigid plastic cisterns. At the maturation room, seawater was used to fill six 2.2-m^3 maturation tanks, while 5-m^3 seawater was stored in reservoirs in order to contain the discharge as recycled water (before pumping) or surplus seawater (to further 'keep the level') respectively. In the

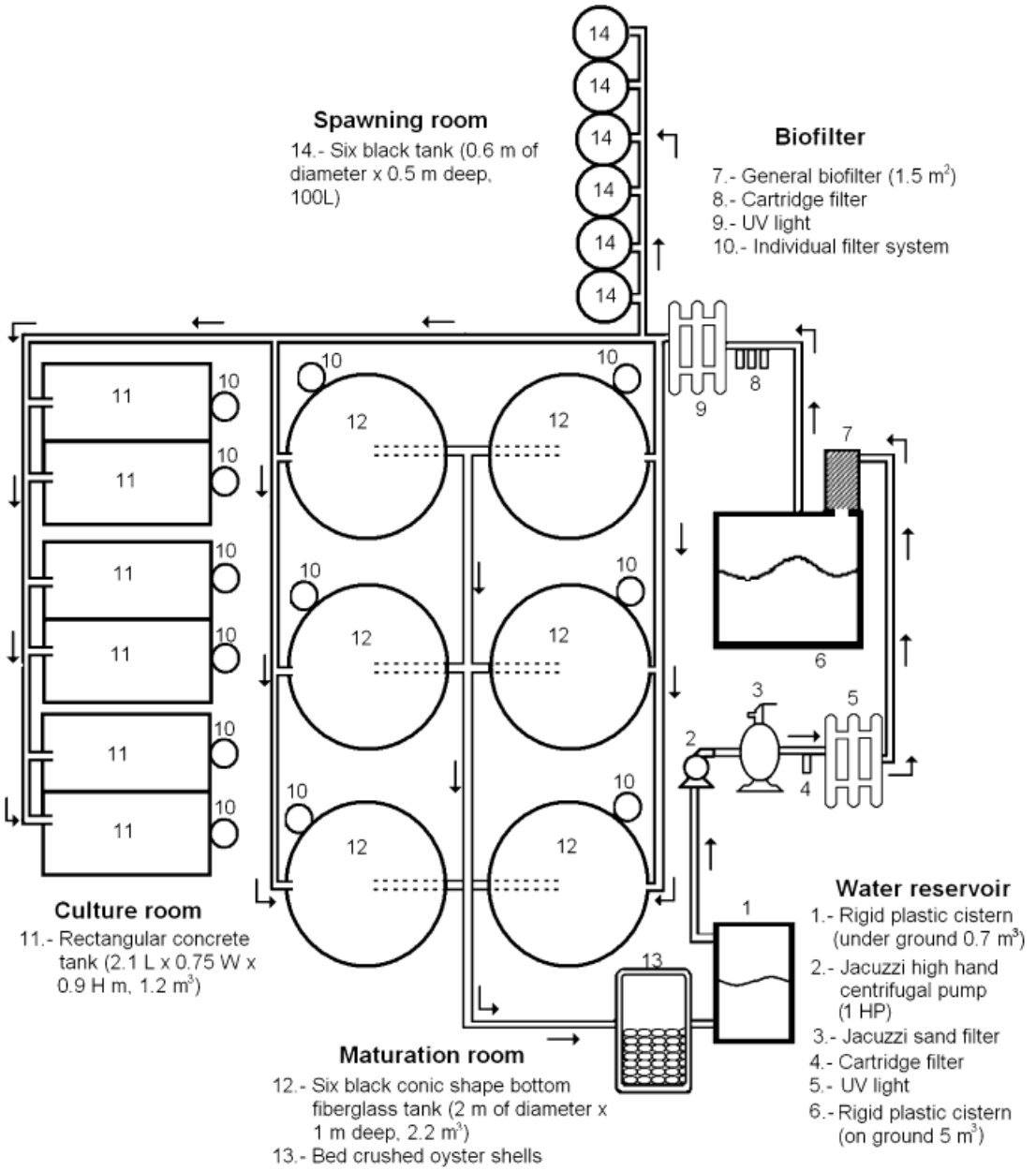

Figure 1 Schematic diagram of the closed recirculation system (not drawn to scale).

culture and maturation room, no sea or freshwater was added after that.

Water was pumped from the underground reservoir using a 1-HP Jacuzzi™ centrifugal pump to a 35-μ Jacuzzi™ sand filter, passed through the cartridge filters and four UV lights. Water was sub sequently sent to the EGBF and stored at the above-ground reservoir, which was thermally isolated by an external thermoplastic covering for further distribution to the CRS tanks by gravity (Copeland *et al.* 2005; Kuhn *et al.* 2007). The above-ground reservoir was supplied with aeration and two portable heaters (2500 W, 220 V, Aquatic Eco-System™, Apopka, FL, USA) set at 28 °C.

Water temperature and salinity were monitored daily using a mercury thermometer (10–100 °C) and a refractometer (model SR6; Aquatic Ecosystems). Ammonia (NH_3), nitrite (NO_2), nitrate (NO_3) and pH were measured weekly (Peixoto *et al.* 2005; Gandy *et al.* 2007) on site using a LaMotte Seawater kit,

Model AQ-4 (Chestertown, MD, USA). Water samples were obtained from each culture or maturation tank. Daily records of water temperature and salinity were grouped by week. During the pre-adult to adult and maturation stages, water samples were collected every 20 days and analysed for ion concentration at the Water Quality Laboratory (Laboratorio de Calidad Ambiental de Tamaulipas) using a Dionex™ (Sunnyvale, CA, USA) Model 10 ion chromatograph for chlorine, ion chromatography coupled with inductively coupled plasma mass spectrometry using a Shimadzu™ (Shimadzu, Kyoto, Japan) HPLC System for magnesium, sodium, potassium, calcium, boron and zinc.

Culture room

This area consisted of six rectangular concrete tanks (2.1 × 0.75 × 0.90 m) covered with white fibre glass resin. The tanks had a 1% sloped bottom to one end (0.75 m) and a lateral discharge. Each tank was supplied with aeration, 1.2 m^3 of brackish water and an IBS. Six fluorescent lights (39 W, cool white) were located 1.5 m above the water surface, and water temperature was set at 28 °C with an electronic sensor adapted to an air conditioning heat pump unit (60 000 BTU's air conditioning).

Maturation room

The walls, tanks and ceiling of this area were painted black and contained six conic-shaped bottom fibreglass tanks (2 m diameter, 1 m height and 1% of centre inclination) and a central drain. Each tank was supplied with aeration, 2.2 m^3 of seawater, IBS and ASIB. Eight fluorescent lights (39 W, cool white) were located 2 m above the tanks to control the photoperiod (12 h L:D) in accordance with Gandy *et al.* (2007). Water temperature was fixed at 28 °C.

Spawning room

The walls, tanks and ceiling of this area were painted black and contained six plastic tanks (0.6 m diameter, 0.5 m height). Each tank was supplied with aeration and 100 L of seawater, and no water circulation was allowed. Room temperature was fixed at 28 °C.

Shrimp source

Specific pathogen-free shrimp nauplii III–IV of *Litopenaeus vannamei* (Boone) were obtained in two batches (2004 and 2005) from a commercial shrimp hatchery (Unidad Marina, La Pesca) and transported in seawater (20 °C, pH 7.8–8.2, 32 g L^{-1} salinity) in a polystyrene box to the shrimp culture facility equipped with the CRS. Nauplii were stocked in two concrete culture tanks with seawater (32 g L^{-1}, 27 °C, pH 8.2) and supplied with aeration as described by Kuhn *et al.* (2007). *Chaetoceros muelleri* (Lemmerman), *Tetraselmis suecica* (Kylin), *Artemia franciscana* (Kellogg) nauplii (Microfeast®; Bartsville, USA) were used as food for protozoa 1 to postlarvae 1 (Aguirre-Guzmán, Vázquez-Juárez & Ascencio-Valle 2001). Early juvenile shrimp were fed *ad libitum* with commercial pellets containing 35% crude protein (Anderson Clayton, Mexico City, Mexico), *A. franciscana* and *Spirulina* sp., until they reached the juvenile stage.

Experimental design

Shrimp growth from juvenile to pre-adult, pre-adult to adult and their subsequent gonadal maturation was tested in the CRS in two consecutive 1-year period (2004–2005 and 2005–2006) trials, labelled as T_1 and T_2 respectively. Juvenile, pre-adult and adult shrimp reared during both the trials were periodically collected, measured and weighed.

Juvenile shrimp with an initial mean body weight of 1.48 ± 0.3 g were grown for 10 weeks at a density of 128–140 shrimp per tank (118–129 g m^2) (Perez-Rostro & Ibarra 2003) with an initial salinity of 15 g L^{-1} (Kuhn *et al.* 2007). Shrimp growth was calculated weekly according to Ricque, Abdo, Cruz, Cuzon, Cousin, Aquacop and Pike (1998), and Molina-Poveda and Morales (2004). Shrimp were fed *ad libitum* with commercial pellets containing 35% crude protein (Anderson Clayton). Excess feed, moults and faeces were removed at 08:00 hours by siphoning, and all dead shrimp were counted and removed (Gandy *et al.* 2007). All water discharges were sock-filtered (at 250 μ) and returned to the corresponding tanks. Water level in each tank was maintained using the surplus brackish water stored in the reservoirs.

Pre-adult shrimp phases, at 20.1 ± 0.8 g (T_1) or 22.2 ± 1.5 g (T_2) of the initial mean body weight, were grown for 7 weeks at a density of 40 shrimp per tank (255–280 g m^2) (Perez-Rostro & Ibarra 2003). Initial water salinity was 32 g L^{-1}. Shrimp survival and growth rate were calculated weekly as described above. Shrimp were fed with commercial pellets containing 35% crude protein (Anderson Clayton) at a rate of 4% of body weight per day, and frozen squid and oyster at a rate of 12% of the total body weight per day (Wouters, Zambrano, Espin, Cal-

deron, Lavens & Sorgeloos 2002; Fegan & Wouters 2004). Daily shrimp maintenance was performed according to the previous description. The water level in each tank was maintained using the surplus seawater stored in the reservoirs.

Shrimp maturation

Sixty female (34.4 ± 5.2 and 31.3 ± 7.2 g of mean body weight) and male shrimp (30.7 ± 5.4 and 28.8 ± 5.3 g of mean body weight) were selected in T_1 and T_2 respectively [ratio 1:1, at a density of 10 females per tank (100–110 g m^2)] (Wyban, Martinez & Sweeney 1997). The maturation phase lasted for 10 weeks at 32 g L^{-1} water salinity. All the females were identified with numbered plastic tags (National Band & Tag, Newport, NY, USA), to record maturation or spawning stages. In T_1, the females were unilaterally ablated by cauterization (Gandy *et al.* 2007), while in T_2, non-ablated female maturation was assessed. All shrimp (males and females) were fed frozen squid and oysters (similar to the pre-adult diet), and a commercial maturation feed (Ziegler Bros., Gardners, PA, USA) at 4% of the total wet shrimp biomass per day (Fegan & Wouters 2004). Females were inspected every night from 18:00 to 20:00 hours to assess the stage of ovarian development. Female shrimp with clear developed ovaries (stage III, well-developed olive-green ovaries, which occupied all the available space in the body cavity) (Peixoto *et al.* 2005) were removed and placed in individual spawning tanks. The next day at 08:00 hours, females were returned to their original tanks (Peixoto *et al.* 2005; Gandy *et al.* 2007). The spawning rate and apparent hatching rate were evaluated using the procedure described by Huang, Jiang, Lin, Zhou and Le (2008) and Nimrat, Sangnawaiuj and Vuthiphandchai (2005) respectively. Random samples from the spawning tank were used to determin the quality and total egg numbers (Babu, Kitto, Ravi & Marian 2001; Peixoto *et al.* 2005).

Statistical analysis

Growth, survival (juvenile–pre-adult–adult), maturation and spawn rates were evaluated by ANOVA and Student's *t*-test using commercial software (STATISTICA data analysis software system, version 7. 2004). The seawater values for temperature, pH, salinity, NH_3, NO_3, NO_2 and ion concentration were analysed using a similar statistical protocol (Kuhn *et al.* 2007).

Results

Water quality parameters

The general water quality parameters detected on the CRS (temperature, pH, salinity, NH_3, NO_2 and NO_3) are presented in Table 1. No significant differences ($P > 0.05$) were detected during the juvenile to pre-adult growth phase, in the pre-adult to adult growth phase or in the maturation phase in any of the trials.

During the juvenile to pre-adult growth phase, the mean temperature ranged from 27 to 28.5 °C, and the pH values started at 7.5 and ended at 7.9. Salinity showed a gradual increase from 15 to 21 or 20 g L^{-1}, T_1 and T_2 respectively. Also, the mean NO_2 started at

Table 1 General water quality parameters (mean ± SD) from during different shrimp (*Litopenaeus vannamei,* Boone) growth phases for a closed recirculation system (CRS) with zero water exchange, during two consecutive trials

		Shrimp growth phases (week of culture)		
Water parameter	Trail	Juvenile to pre-adult (17 week)	Pre-adult to adult (7 week)	Maturation (10 week)
Temperature (°C)	T_1	27 ± 0 to 28.5 ± 0	26 ± 0 to 28 ± 0.1	28 ± 0 to 28 ± 0.5
	T_2	28 ± 0 to 28 ± 0.2	28 ± 0 to 28 ± 0.1	28 ± 0 to 28 ± 0.2
pH	T_1	7.5 ± 0.1 to 7.9 ± 0	7.6 ± 0 to 8.1 ± 0	6.3 ± 0.05 to 8.05 ± 0.02
	T_2	7.5 ± 0.1 to7.9 ± 0	7.8 ± 0.2 to 8.3 ± 0	6.3 ± 0.05 to 8.05 ± 0.02
Salinity (g L^{-1})	T_1	15 ± 1 to 21 ± 1	32 ± 1 to 34 ± 1	32 ± 0.5 to 34 ± 0.8
	T_2	15 ± 1 to 20 ± 1	32 ± 1 to 35 ± 1	32 ± 0 to 34 ± 0
NO_2 (mg L^{-1})	T_1	0.05 ± 0 to 0.68 ± 0.1	0.05 ± 0 to 0.6 ± 0.08	0.02 ± 0 to 0.5 ± 0
	T_2	0.05 ± 0 to 0.68 ± 0.1	0.05 ± 0 to 0.7 ± 0.05	0.02 ± 0 to 0.6 ± 0
NO_3 (mg L^{-1})	T_1	2 ± 0 to 9 ± 1.7	2.5 ± 0.8 to 4.2 ± 0.3	2 ± 0 to 5.7 ± 2.3
	T_2	2 ± 0 to 9 ± 1.7	2.8 ± 0.9 to 4.7 ± 0.3	2 ± 0 to 5.3 ± 0.3
NH_3 (mg L^{-1})	T_1	0 ± 0 to 0.05 ± 0.03	0 ± 0 to 0.05 ± 0.01	0 ± 0 to 0.03 ± 0.02
	T_2	0 ± 0 to 0.08 ± 0.01	0 ± 0 to 0.05 ± 0.01	0 ± 0 to 0.06 ± 0.01

T_1, trial 1 (2004–2005); T_2, trial (2005–2006).

0.05 mg L^{-1} and increased to 0.68 mg L^{-1}. Nitrate displayed an increase along the trials, with a higher level at 9 mg L^{-1}. NH_3 ranged from 0 to 0.05 or 0.08 mg L^{-1}, T_1 and T_2 respectively.

The temperature in the pre-adult to adult phase was similar to that from the juvenile to pre-adult growth phase. The mean pH values had a decreasing tendency, starting at 8.1–8.3 and ending at 7.6–7.8 in both the trials. Salinity ranged from 32 to 34 or 35 g L^{-1}, T_1 and T_2 respectively. NO_2 started at 0.05 mg L^{-1} and increased to 0.6 and 0.7 mg L^{-1}. NO_3 showed similar fluctuations between the trials (data not shown) without significant differences ($P > 0.05$) and ranged from 2.5–2.8 to 4.2–4.7 mg L^{-1}. The mean NH_3 levels started at 0 and ended at 0.05 mg L^{-1} in both the trials.

The temperature during the maturation shrimp growth phase was 28 °C. The mean pH decreased during both the trials, starting at 8.05 and ending at 6.3. Salinity ranged from 32 to 34 g L^{-1}. NO_2 and NO_3 fluctuated between the trials, ranging from 0.02 and 2 mg L^{-1} to 0.6 and 5.7 mg L^{-1} respectively. In both the trials, the mean NH_3 levels started at 0 and ended at 0.03 or 0.06 mg L^{-1}.

Some variations in seawater ion concentrations (Table 2) were observed between the trials (T_1 and T_2), but no significant differences were detected compared with initial seawater ($P > 0.05$). Only a significant difference in the concentration of phosphorus was observed in both the trials compared with initial seawater ($P > 0.05$).

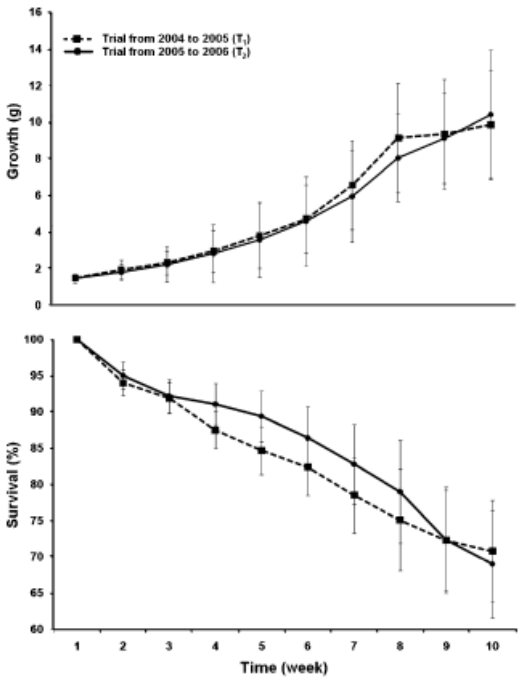

Figure 2 Growth (g) and survival (%) from juvenile to pre-adult shrimp (*Litopenaeus vannamei*, Boone) during the trials (mean ± SD).

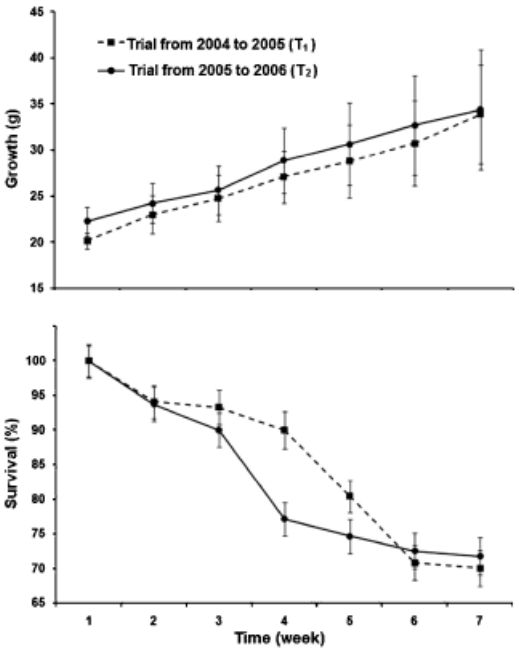

Figure 3 Growth (g) and survival (%) from pre-adult to adult shrimp (*Litopenaeus vannamei*, Boone) during the trials (mean ± SD).

Shrimp growth and survival

A similar growth from juvenile to pre-adult phase was observed in shrimp between T_1 and T_2, but there were no significant differences in the overall weight gain (Fig. 2). In the T_1 growth phase, a higher weight gain ($P > 0.05$) was observed in weeks 7 and 8; while in T_2 there was a higher weight gain ($P > 0.05$) in week 10 (Fig. 2). Growth from the pre-adult to the adult between phase T_1 and T_2 showed significant differences in week 1 ($P > 0.05$). Shrimp in T_2 were 2.11 g heavier than those in T_1 and showed higher weight gain ($P > 0.05$) in weeks 4 and 5 (Fig. 3), but there were no significant differences in the overall weight gain between the trials (T_1 and T_2).

There were no significant differences in the shrimp survival of juvenile to pre-adult and pre-adult to adult in both the trials (Figs 2 and 3). The survival rate in the juvenile to pre-adult phase was 70% and 69% in T_1 and T_2, while in the pre-adult to adult phase it was 70% and 71% respectively.

Spawning rate and apparent hatching rate

The spawning rate showed no significant differences ($P > 0.05$) between trials (10.6 ± 2% and 9.1 ± 3%). In both cases, spawned eggs were lysed after 6 h. However, in the second trial, some mature females were placed in spawning containers filled with the CRS unused seawater, where the apparent hatching rate was calculated from 91.7% to 92.9%.

Discussion

The aquaculture industry demands good-quality organisms for culture. This represents an opportunity for CRS, whose results in pilot studies suggest that they can be used commercially as biosecure culture areas (Kaiser *et al.* 1998; Cytryn *et al.* 2003; Delabbio *et al.* 2003; Copeland *et al.* 2005; Gandy *et al.* 2007; Kuhn *et al.* 2007). Properly designed CRS provide a suitable culture environment for shrimp growth and offer a stable water quality with minimum fluctuations of physicochemical parameters such as temperature, salinity, pH, ammonia, nitrite and nitrate (Kaiser *et al.* 1998; Cytryn *et al.* 2003).

Water quality and parameters

Temperature range from 25 to 30 °C is reported for shrimp growth and maturation of *L. vannamei*, *Farfantepenaeus paulensis* (Pérez Farfante) and *Farfantepenaeus aztecus* (Ives) (Boyd 2001; Peixoto *et al.* 2005; Gandy *et al.* 2007; Kuhn *et al.* 2007). Similar temperature ranges were observed in the present work (26–28.5 °C in the juvenile to adult shrimp growth, and 28 °C in the maturation phase), and did not appear to have a negative effect on shrimp growth (Figs 2 and 3), maturation, spawn or hatching rate.

Salinity for growth, brood-stock development and maturation for *L. vannamei*, *F. paulensis*, *Farfantepenaeus brasiliensis* (Latreille) and *F. aztecus* has been registered from 1 to 35 (Brito, Chimal & Rosas 2000; Boyd 2001; Otoshi, Arce & Moss 2003; Mishra, Samocha, Patnaik, Speed, Gandy & Abdul-Mehdi 2008) and a high level, such as 50 g L^{-1}, for *L. vannamei* (Perez-Velazquez, González-Félix & Jaimes-Bustamante 2007). Li, Chen, Zeng, Chen, Yu, Lai and Qin (2007) emphasize that the optimal salinity for *L. vannamei* growth is still controversial and show that shrimp are less vulnerable to environmental stress at 15–35 g L^{-1} of salinity. In this CRS, the juvenile to pre-adult phase developed at 15 g L^{-1}, assuming that this is the salinity in the environment of the costal lagoons where shrimp post-larvae and juvenile grow (Martínez-Córdova 1999), and that was reported as the salinity level with a better growth performance for shrimp (Lin & Chen 2003). Later development was observed in the common range from 32 to 35 g L^{-1}. From these findings, salinity levels did not play any detrimental role in these trials.

NH_3, NO_2 and NO_3 were used as water quality indicators and CRS failure (Kaiser *et al.* 1998; Boyd 2001; Decamp *et al.* 2007). In this study, NO_2 and NH_3 levels (Table 1) were lower than the toxic levels reported for shrimp juvenile, pre-adult and maturing adults (Boyd 2001; Magallón-Barajas, Servín-Villegas, Portillo-Clark, García-Mosqueda & López-Moreno 2006; Mishra *et al.* 2008). Those results suggest an effective water-purification process and the establishment of the bacterial biofilter necessary for nitrification (Cytryn *et al.* 2003; Perez-Velazquez, González-Félix, Gómez-Jiménez, Davis & Miramontes-Higuera 2008).

In this CRS, the pH for juvenile to adult remained between 7.5 and 8.3 levels (Table 1). Similar pH levels have been documented for different shrimp species and their developmental stages (Avnimelech, Kochva & Diab 1994; Laramore, Laramore & Scarpa 2001; McGraw, Davis, Teichert-Coddington & Rouse 2002; Cohen, Samocha, Fox, Gandy & Lawrence 2005). A decrease in pH from 8 to 6.3 was observed during the maturation phase. Lekang (2007) suggests that the pH decrease for CRS in which seawater was to be used for a long time is due to the bacterial biofilter, which generates carbon dioxide and some acid products, which drive the pH downwards. This process may be solved by the use of calcium carbonate, as suggested by Boyd (2001).

Growth of juveniles to adults and maturation

There is no documented evidence on the use of a zero-exchange CRS on an entire shrimp life cycle (egg to egg). Otoshi *et al.* (2003) report the lack of information about brood-stock growth, and shrimp reproductive performance on recirculation aquaculture systems (RAS). They compared the shrimp growth and reproductive performance in two trials, where shrimp were cultured from market (20 g) to the brood-stock size (40–60 g) in RAS vs. a flow-through earthen pond. However, they did not indicate whether the development of shrimp was accomplished in a CRS with no seawater exchange or

Table 2 Trace elements level (mg L^{-1}) of seawater from pre-adult to maturation stage (100 total days)

	Initial seawater	T_1	T_2
Cl	20 122.70 ± 85.56	19 441.38 ± 501.95	19 253.18 ± 571.48
Mg	2084.00 ± 70.71	1993.78 ± 64.45	2005.84 ± 72.78
Na	6083.70 ± 2.83	6256.52 ± 133.44	6303.80 ± 101.62
K	1142.50 ± 7.78	1303.73 ± 184.19	1266.86 ± 123.68
Ca	546.20 ± 2.83	663.22 ± 58.86	672.32 ± 60.44
B	7.25 ± 0.07	7.68 ± 0.28	7.72 ± 0.40
P	0.35 ± 0.07	9.90 ± 5.01*	12.18 ± 2.70*
Zn	0.10 ± 0.00	0.25 ± 0.13	0.25 ± 0.06

T_1, trial (2004–2005); T_2, trial (2005–2006).
*Differences between periods ($P > 0.05$) no statistical significance among trials ($P > 0.05$).

replenishment. Gandy (2004) developed *F. aztecus* from a juvenile to the reproductive size in a CRS, but there was no mention of seawater exchange in this report.

The observed shrimp growth from juvenile to pre-adult was similar between the trials (T_1 and T_2) without significant differences, and at 10 weeks of culture, the mean individual weight was 9.9 ± 2.1 and 10.4 ± 3.5 g respectively. The pre-adult to adult mean weight of shrimp was statistically lower in weeks 1, 4 and 5 ($P > 0.05$) in T_1 compared with the same weeks of T_2 (Fig. 3). After 7 weeks of the culture, the final mean weight of shrimp was 33.91 ± 5.3 in T_1 and 34.38 ± 6.4 g in T_2. Similar growth results have been reported by Tacon, Cody, Conquest, Divakaran, Forster & Decamp (2002), Cytryn *et al.* (2003), Delabbio *et al.* (2003), Copeland *et al.* (2005), Gandy (2004), Gandy *et al.* (2007), Kuhn *et al.* (2007) and Green (2008).

Our results clearly reveal a similar growth pattern for juvenile to pre-adult and pre-adult to adult *L. vannamei* in both the trials (Figs 2 and 3). This suggests the possibility of obtaining similar results in water quality and shrimp growth in a CRS with 'no brackish or seawater exchange' during different periods of culture. It also provides the possibility of using this system for research and/or in the commercial shrimp industry as a biosecure area for the development of SPF shrimp or specific genetic lines from a nauplii to an adult.

Maturation, spawning and hatching

Different internal (brood-stock source and size, nutrition and genetics) and external factors (tank shape and size and environmental conditions) may affect the shrimp reproduction (Tacon *et al.* 2002; Racotta, Palacios & Ibarra 2003; Aktaş & Kumlu 2005; Malpartida & Vinatea 2007). Babu *et al.* (2001) showed that fluctuations in water quality parameters may inhibit shrimp maturation. However, *L. vannamei* is reported to be a tolerant species, able to adapt to fluctuations in salinity, pH and dissolved oxygen levels (Rosenberry 1999). *Penaeus monodon*'s (Fabricius) female maturation, spawning and hatching have been observed at a pH of 7.5 ± 0.5 (Babu *et al.* 2001). In this study, no significant differences were found between ablated and non-ablated *L. vannamei* females regarding maturation between the trials. Also, the spawning rate shows no significant differences between the trials and was similar to that reported by Sangpradub, Fast, Piyatiratitivorakuland and Menasveta (1994) and Gandy *et al.* (2007). This suggests that the water quality fluctuations observed in this CRS do not present a threat at the developmental and maturation stages. This is the first report on the use of a CRS with zero water exchange in shrimp reproductive areas.

In both the trials, spawned eggs were lysed after 6 h, and only with unused fresh seawater was it possible to obtain shrimp nauplii (with an apparent hatching rate of 91.7–92.9%). A similar hatching rate was obtained by Babu *et al.* (2001) and Nimrat *et al.* (2005). Van Wyk and Scarpe (1999) found that the ratio of the divalent ions (Ca^{+2} and Mg^{+2}) to the monovalent ions (Na+ and K+) is critical for shrimp reared at a low salinity. Lynn and Clark (1987) suggest that egg jelly-releasing precursors in *F. aztecus* were inhibited for a possible deficiency of Mg^{+2} in the seawater. It could be surmised that the ion imbalances (Cl^-, Mg^{+2}, P, Zn, B or Na) reported in this study are due to the decaying conditions of the seawater used for such a long time period, and might be a cause of the failure to complete the life cycle of *L. vannamei* in a CRS, with a no seawater exchange or replenishment. It appears that there is a new cluster of opportunities to work with, at the spawn and hatching stages.

This is the first report on the use of a CRS with zero water exchange in different shrimp phases (juvenile, pre-adult, adult and maturation) with similar results (without significant differences) during 2 consecutive years. These results show the possibility of using this system for shrimp culture, increasing biosecurity against disease and for rearing organisms with specific genetic lines with faster growth, better survival and feed conversion rates, or increased disease resistance. Further investigations are required on this type of technology in order to complete the shrimp life cycle in a CRS.

Acknowledgments

We thank the UAT and Programa de Mejoramiento del Profesorado (PROMEP) for providing the funds for this research. The Universidad Autonoma de Nuevo Leon, Facultad de Ciencias Biologicas, supported this work with technical assistance. Laboratorio de Calidad Ambiental de Tamaulipas performed and paid the costs of ion analysis of seawater samples.

References

Aguirre-Guzmán G., Vázquez-Juárez R. & Ascencio-Valle F. (2001) Differences in the susceptibility of American white shrimp larvae substages (*Litopenaeus vannamei*) to four *Vibrio* species. *Journal of Invertebrate Pathology* **78**, 215–219.

Aktaş M. & Kumlu M. (2005) Gonadal maturation and spawning in *Penaeus semisulcatus* de Hann, 1844 by hormone injection. *Turkish Journal of Zoology* **29**, 193–199.

Avnimelech Y., Kochva M. & Diab S. (1994) Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. *The Israeli Journal of Aquaculture* **46**, 119–131.

Babu M., Kitto M.R., Ravi C. & Marian M.P. (2001) Factors determining spawning success in *Penaeus monodon* Fabricius. 15 Naga. *The ICLARM Quarterly* **24**, 13–15.

Boyd C.E. (2001) Consideraciones sobre la calidad del agua y del suelo en cultivos de camarón. In: *Métodos para mejorar la camaronicultura en Centroamérica* (ed. by M.C. Haws & C.E. Boyd), pp. 1–30. Tr. Ochoa-Moreno E. Ed. Imprenta. 1er edición, Managua, Nicaragua.

Brito R., Chimal M.E. & Rosas C. (2000) Effect of salinity in survival, growth, and osmotic capacity of early juveniles of *Farfantepenaeus brasiliensis* (decapoda: penaeidae). *Journal of Experimental Marine Biology and Ecology* **244**, 253–263.

Cohen J., Samocha T.M., Fox J.M., Gandy R.L. & Lawrence A.L. (2005) Characterization of water quality factors during intensive raceway production of juvenile *Litopenaeu vannamei* using limited discharge and biosecure management tools. *Aquacultural Engineering* **32**, 425–442.

Copeland K.A., Watanabe W.O. & Dumas C.E. (2005) Economic evaluation of a small-scale recirculating system for ongrowing of captive wild black sea bass *Centropristis striata* in North Carolina. *Journal of the World Aquaculture Society* **36**, 489–497.

Cytryn E., Gelfand I., Barak Y., Rijn J.V. & Minz D. (2003) Diversity of microbial communities correlated to physiochemical parameters in a digestion basin of a zero discharge mariculture system. *Environmental Microbiology* **5**, 55–63.

Decamp O., Conquest L., Cody J., Forster I. & Tacon A.G.J. (2007) Effect of shrimp stocking density on size-fractionated phytoplankton and ecological groups of ciliated protozoa within zero-water exchange shrimp culture systems. *Journal of the World Aquaculture Society* **38**, 395–406.

Delabbio J., Murphy B.R., Johnson G.R. & Hallerman E.M. (2003) Characteristics of the recirculation sector of finfish aquaculture in the United States and Canada. *International Journal of Recirculating Aquaculture* **4**, 5–23.

FAO Fisheries Department (2004) Series title: state of world fisheries and aquaculture (SOFIA).

Farzanfar A. (2006) The use of probiotics in shrimp aquaculture. *Federation of European Microbiological Societies (FEMS) Immunology and Medical Microbiology* **48**, 149–158.

Fegan D. (2001) Update: shrimp farming in Southeast Asia. *The Global Aquaculture Advocate* **4**, 81.

Fegan D. & Wouters R. (2004) Technical considerations in the development of feeds for shrimp larve and broodstock. In: *Avances en Nutrición Acuícola VII. Memorias del VII Simposium Internacional de Nutrición Acuícola*, 16–19 Noviembre, 2004, Hermosillo, Sonora, México, (ed. by Suarez, L.E. Cruz, D. Ricque Marie, M.G. Nieto López, D. Villarreal, U. Scholz & M. González), pp. 143–164. Universidad Autonoma de Nuevo Leon, Mexico.

Gandy R.L. (2004) *Investigations into the reproductive performance and larval rearing of the brown shrimp, Farfantepenaeus aztecus, using closed recirculating systems.* PhD Thesis, Wildlife and Fisheries Sciences, Texas A&M University, TX, USA.

Gandy R.L., Samocha T.M., Masser M.P., Fox J.M., Ali A.M.S., Gatlin D.M. & Speed M. (2007) The effect of unilateral eyestalk ablation and diet on the reproductive performance of wild-caught *Farfantepenaeus aztecus* (Ives, 1891) using a closed recirculating maturation system. *Aquaculture Research* **38**, 580–587.

Gelfand I., Barak Y., Even-Chen Z., Cytryn E., Rijn J.V., Krom M.D. & Neori A. (2007) A novel zero discharge intensive seawater recirculating system for the culture of marine fish. *Journal of the World Aquaculture Society* **34**, 344–358.

Green B.W. (2008) Stocking strategies for production of *Litopenaeus vannamei* (Boone) in amended freshwater in inland ponds. *Aquaculture Research* **39**, 10–17.

Huang J.H., Jiang S.G., Lin H.Z., Zhou F.L. & Le Y. (2008) Effects of dietary highly unsaturated fatty acids and astaxanthin on the fecundity and lipid content of pond-reared *Penaeus monodon*(Fabricius) broodstock. *Aquaculture Research* **39**, 240–251.

Kaiser H., Paulet T.G. & Endemann F. (1998) Water quality fluctuations in a closed recirculating system for the intensive culture of the guppy, *Poecilia reticulata* (Peters). *Aquaculture Research* **29**, 611–615.

Kamstra A., Van der Heul J.W. & Nijhof M. (1998) Performance and optimization of trickling filters on eel farms. *Aquacultural Engineering* **17**, 175–192.

Kuhn D.D., Boardman G.D., Craig S.R., Flick G.J. & McLean E. (2007) Evaluation of tilapia effluent with ion supplementation for marine shrimp production in a recirculating aquaculture system. *Journal of the World Aquaculture Society* **38**, 74–84.

Laramore A., Laramore C.R. & Scarpa J. (2001) Effect of low salinity on growth and survival of the Pacific white

shrimp *Litopenaeus vannamei*. *Journal of the World Aquaculture Society* **32**, 385–392.

Lekang O.I. (2007) *Aquaculture Engineering*, pp. 37–130. Blackwell publishing, Oxford, UK.

Li E.C., Chen L.Q., Zeng C., Chen X.M., Yu N., Lai Q. & Qin J.G. (2007) Growth, body composition, respiration and ambient ammonia nitrogen tolerance of the juvenile white shrimp, *Litopenaeus vannamei*, at different salinities. *Aquaculture* **265**, 385–390.

Lightner D.V. (2003) *The penaeid shrimp viral pandemics due to IHHNV, WSSV, TSV and YHV: history in the Americas and current status*. Proceedings of the thirty-second UJNR Aquaculture Panel Symposium, Aquaculture and Pathobiology of Crustacean and Other Species, November 17–18 and 20, Santa Barbara, CA, USA.

Lin Y.C. & Chen J.C. (2003) Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Aquaculture* **224**, 193–201.

Lobo R., Revah S. & Viveros G. (1999) An analysis of a trickle bed bioreactor: carbon disulfide removal. *Biotechnology and Bioengineering* **63**, 98–109.

Lynn J.W. & Clark W.H. Jr. (1987) Physiological and biochemical investigations of the egg jelly release in *Penaeus aztecus*. *Biology Bulletin* **173**, 451–460.

Magallón-Barajas F., Servín-Villegas R., Portillo-Clark G., García-Mosqueda J. & López-Moreno B. (2006) Daily variation in short-term static toxicity of unionized ammonia in *Litopenaeu vannamei* (Boone) postlarvae. *Aquaculture Research* **37**, 1406–1412.

Malpartida J. & Vinatea L. (2007) Monitoramento do crescimento de juvenis de *Farfantepenaeus paulensis* (Perez-Farfante, 1967) com vistas a um futuro repovoamento da Lagoa de Ibiraquera, Imbituba, SC. *Biotemas* **20**, 37–45.

Martínez-Córdova L.R. (1999). *Cultivo de camarones Peneidos: Principios y Prácticas*. Primera Edición, A.G.T. Editor, S.A. México.

McGraw W., Davis D., Teichert-Coddington D. & Rouse D. (2002) Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: influence of age, salinity endpoint, and rate of salinity reduction. *Journal of the World Aquaculture Society* **33**, 78–84.

Mishra J.K., Samocha T.M., Patnaik S., Speed M., Gandy R.L. & Abdul-Mehdi A. (2008) Performance of an intensive nursery system for the Pacific white shrimp, *Litopenaeus vannamei*, under limited discharge condition. *Aquacultural Engineering* **38**, 2–15.

Molina-Poveda C. & Morales M.E. (2004) Use of a mixture of barley-based fermented grains and wheat gluten as an alternative protein source in practical diets for *Litopenaeus vannamei* (Boone). *Aquaculture Research* **35**, 1158–1165.

Nimrat S., Sangnawaiuj T. & Vuthiphandchai V. (2005) Preservation of black tiger shrimp *Penaeus monodon* spermatophores by chilled storage. *Journal of the World Aquaculture Society* **36**, 76–86.

Otoshi C.A., Arce S.M. & Moss S.M. (2003) Growth and reproductive performance of broodstock shrimp reared in a biosecure recirculating aquaculture system versus a flow-through pond. *Aquacultural Engineering* **29**, 93–107.

Peixoto S., Cavalli R.O. & Wasielesky W. (2005) Recent developments on broodstock maturation and reproduction of *Farfantepenaeus paulensis*. *Brazilian Archives of Biology and Technology* **48**, 997–1006.

Perez-Rostro C. & Ibarra A.M. (2003) Quantitative genetic parameter estimates for size and growth rate traits in Pacific white shrimp, *Penaeus vannamei* (Boone 1931) when reared indoors. *Aquaculture Research* **34**, 543–553.

Perez-Velazquez M., González-Félix M.L. & Jaimes-Bustamante F. (2007) Growth of *Litopenaeus vannamei* raised at extreme salinities. *Panorama Acuicola Magazine* **13**, 46–53.

Perez-Velazquez M., González-Félix M.L., Gómez-Jiménez S., Davis D.A. & Miramontes-Higuera N. (2008) Nitrogen budget for a low-salinity, zero-water exchange culture system: II. Evaluation of isonitrogenous feeding of various dietary protein levels to *Litopenaeus vannamei* (Boone). *Aquaculture Research* **39**, 995–1004.

Racotta I.S., Palacios E. & Ibarra A.M. (2003) Shrimp larval quality in relation to broodstock condition. *Aquaculture* **227**, 107–130.

Ricque D., Abdo M.I., Cruz L.E, Cuzon G., Cousin M., AQUACOP & Pike I.H. (1998) Raw material freshness, a quality criterion for fish meal fed to shrimp. *Aquaculture* **165**, 95–109.

Rosenberry B. (1999) World Shrimp Farming 1999. *Shrimp News International, San Diego California* **12**, 1–31.

Samocha T.M, Lopez I.M., Jones E.R., Jackson S. & Lawrence A.L. (2004) Characterization of intake and effluent waters from intensive and semi-intensive shrimp farms in Texas. *Aquaculture Research* **35**, 321–339.

Sangpradub S., Fast A.W., Piyatiratitivorakuland S. & Menasveta P. (1994) Effects of different feeding regimes on ovarian maturation and spawning of pond-reared giant tiger prawn in Thailand. *Aquaculture International* **2**, 49–58.

Sharrer M.J., Summerfelt S.T., Bullock G.L., Gleason L.E. & Taeuber J. (2005) Inactivation of bacteria using ultraviolet irradiation in a recirculating salmonid culture system. *Aquacultural Engineering* **33**, 135–149.

Tacon A.G.J., Cody J.J., Conquest L.D., Divakaran S., Forster I.P & Decamp O.E. (2002) Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquaculture Nutrition* **8**, 121–137.

Tomoda T., Fushimi H. & Kurokura H. (2005) Performance of a closed recirculation system for larviculture of red sea bream, *Pagrus major*. *Fisheries Science* **71**, 1179–1181.

Van Wyk P. & Scarpe J. (1999) Water quality requirements and management. In: *Farming Marine Shrimp in Recirculating Freshwater Systems, A Practical Manual* (ed. by P. Van Wyk, M. Davis-Hodgkins, R. Laramore, K.L.

Main, J. Mountain & J. Scarpa), pp. 141–161. Harbor Branch Oceanographic Institution, Florida State University and USDA's Agricultural Research Service, FL, USA.

Wouters R., Zambrano B., Espin M., Calderon J., Lavens P. & Sorgeloos P. (2002) Experimental broodstock diets as partial fresh food substitutes in white shrimp *Litopenaeus vannamei* B. *Aquaculture Nutrition* **8**, 249–256.

Wyban J., Martinez G. & Sweeney J. (1997) Adding paprika to *Penaeus vannamei* maturation diet improves nauplii quality.*World Aquaculture* **28**, 59–62.

Xiongfei W., Zhidong Z., Deshang L., Kangmei C., Zhuanshang T., Liegang S., Kaichong X. & Bailin G. (2005) Closed recirculating system for shrimp-mollusk polyculture. *Chinese Journal of Oceanology and Limnology* **23**, 461–468.