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Effect of salinity on natural community and production of *Litopenaeus vannamei* (Boone), within experimental zero-water exchange culture systems

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Abstract

Recent efforts have been made to culture marine shrimp in systems operating under low or zero-water exchange and with decreased water salinity. The aim of this study was to investigate the impact of various salinity levels on qualitative and quantitative characteristics of the natural community and, more particularly, ciliated protozoa, and compare this information with shrimp growth and survival. Tanks with 9‰ salinity were characterized by a higher pH, but also by a significantly higher concentration of chlorophyll *a* (Chl *a*) per weight of suspended matter (1.93 ± 0.72 µg Chl *a*/mg TSS) than tanks with 18‰ (1.29 ± 0.68 µg Chl *a*/mg TSS) or 36‰ (1.37 ± 0.61 µg Chl *a*/mg TSS) salinity. Concentrations of ciliates (max 6000 cells mL^{-1}) showed considerable fluctuations over the sampling period, reflecting the impact of water salinity, dynamic interactions between ciliates and their diverse roles within the shrimp production system. There was no significant difference between survival rates of shrimp reared at 9‰, 18‰ or 36‰, but decreasing salinity from 36‰ to 9‰ led to a significant decrease in final shrimp body weight (from 13.40 ± 0.26 g to 10.23 ± 2.72 g). Future work should address the potential of ciliates as an indicator of aquaculture water quality, as is currently being done in the wastewater industry, and the contribution of ciliates as food sources.

Keywords: intensive shrimp aquaculture, *Litopenaeus vannamei*, population dynamics, protists, salinity

Introduction

Recent efforts have been made to culture marine shrimp in systems operating under low or zero-water exchange and with decreased water salinity. Advantages of low water exchange include reduced input of water and waterborne pathogens, and release of nutrient-rich effluent into the environment. Decreasing salinity (1) allows movement of shrimp farms away from potentially contaminated coastal waters (Moya, Lawrence, Collins & Samocha 1999), (2) limits the risk of shrimp diseases such as necrotizing hepatopancreatitis, the development of which is dependent on salinities of 20‰ or higher (Frelier, Loy, Varner, Thompson, Lawrence & Bray 1995), (3) eases waste disposal or treatment (Intrasungkha, Keller & Blackall 1999; Dinçer & Kargi 2001) and (4) facilitates the utilization of effluents for secondary production of agricultural products (Brown & Glenn 1999; Brown, Glenn, Fitzsimmons & Smith 1999).

Most penaeid shrimp are euryhaline species and *Litopenaeus vannamei* (Boone) juveniles have been successfully reared at salinities of 5–35‰ (Sturmer & Lawrence 1989; Bray, Lawrence & Leung-Trujillo 1994; Ponce-Palafox, Martinez-Palacios & Ross 1997). However, decreasing salinities may affect shrimp physiology and water quality parameters such as concentrations of ammonia and nitrite; *L. vannamei* ammonia-N excretion rate is reported to be lower at salinity of 25‰ than at salinities of 10‰ or 40‰ (Jiang, Lawrence, Neill & Gong 2000); and nitrite-N excretion of *Penaeus chinensis* juveniles increases with increased salinity, pH and ambient ammonia-N levels, whereas ammonia-N excretion

of *P. chinensis* juveniles decreases with increased salinity, pH and ambient ammonia-N levels (Chen & Lin 1995).

Even in intensive production systems, shrimp benefit from the natural community present within the culture environment (Moss & Pruder 1995). The natural community observed in intensive shrimp production systems includes a wide range of organisms, i.e. bacteria, phytoplankton, protists, rotifers and nematodes. Among protists, ciliates are known to play an important role in the energy flow of aquatic ecosystems. Ciliates consume bacteria, algae and fungi, and are ingested by rotifers, nematodes and fish larvae (Stoeker & Capuzzo 1990; Curds 1992; Fukami, Watanabe, Fujita, Yamaoka & Nishijima 1999). Furthermore, the relative abundance and diversity of ciliated protozoa have been used as indicators of water quality and ecosystem dynamics (Foissner 1988; Curds 1992). However, their occurrence and role in shrimp production systems have seldom been investigated (Bratvold, Lu & Browdy 1999; Decamp, Conquest, Forster & Tacon 2002).

The aims of this study were to investigate the impact of various salinity levels and associated changes in water quality on qualitative and quantitative characteristics of the ciliated protozoa community, and compare this information with shrimp growth and survival.

Materials and methods

Shrimp production experiments were carried out in the Outdoor Mesocosms Laboratory, consisting of free-standing fibreglass tanks, 1.52 m diameter × 1.37 m height, with a conical bottom (for additional information, see Leber & Pruder 1988). Pacific White Shrimp (*L. vannamei*) of average body weight 1.79–1.89 g were stocked at 50 individuals m^{-2} on 25 May 2000 within tanks (working volume 1300 L) of various salinities, i.e. 36‰ (treatment 1), 18‰ (treatment 2) and 9‰ (treatment 3), with three tanks randomly allocated for each treatment. Shrimp were acclimated down from 36‰ by reducing the salinity by 50% every 12 h. When the desired salinity was achieved, shrimp were maintained at desired salinity for at least 2 weeks before trial stocking.

Water within each tank was continuously mixed and aerated by using disk aerators in the conical bottom of the tank. Air was supplied to all tanks with an EG & G Rotron 5 HP regenerative blower (Saugerties, New York, NY). Salinity was measured by using a temperature-compensated refractometer (Aquatic eco-Systems, Apopka, FL), and freshwater and/or seawater was used as required to replace evaporative losses and maintain salinity at the desired value. Each tank was seeded the day before stocking with 10 L of plankton-rich water from the shrimp holding tanks. Shrimp were fed an OI reference diet with 35.2% crude protein content and 8.9% crude lipid content. All treatments were fed to satiation eight times daily between 2 am and 11 pm. Shrimp were weighed on a bi-weekly basis and at harvest, on 19 July 2000, after 56 days of production.

A pH meter (Model 1001, Senton, Gig Harbor, WA) was used to measure pH in every tank twice a week. Dissolved oxygen and water temperature was measured with a meter (Model 55, Yellow Springs Instruments, Yellow Springs, OH) twice daily at 7 am and 4 pm. Tanks of treatment 1 were sampled each working day whereas tanks of the other two treatments were sampled every 3 working days. Water samples (1 L) were collected with plastic bottles at 1pm and brought back to the laboratory. Subsamples were then taken for measurement of total ammonia, nitrate, nitrite, orthophosphate, total suspended solids (TSS), chlorophyll *a*, and for investigations of ciliated protozoa.

Water chemical analyses were carried out with a QuikChem FIA + 8000 Series (Zellweger Analytics, Lachat, Milwaukee, WI), following methods 30-107-06-1-A (ammonia), 31-107-04-1-C (nitrate and nitrite), and 31-115-01-3-C (orthophosphate) (Lachat 2000). Concentrations of TSS and Chl *a* were determined following the methods of Strickland & Parsons (1972). For TSS, a known volume (10–75 mL) of sample was filtered through a dried, preweighed 25 mm glass fibre filter (Osmonics GC 50), under vacuum suction, washed free of salts, dried at 75 °C for 24 h and weighed on a Sartorius microbalance (model M3P). For Chl *a*, samples (10–25 mL) were filtered through a 0.4-μm polycarbonate filter using vacuum suction (7 mmHg). The filter was extracted in 90% acetone (10% water) for 24 h in the dark at −5 °C. The extracted pigment was measured under suitable dilution using a Turner fluorometer, model 10AU (Turner Designs, Sunnyvale, CA), equipped with filters of excitation 430 nm and emission 663 nm. The difference in fluorescence read against a blank (90% acetone) was used to calculate Chl *a* based on

the characteristic absorbtivity coefficient for the pigment.

Two subsamples (about 50 mL) were taken for enumeration and identification of ciliated protozoa. Ciliates were enumerated from unfixed samples by a modified drop method (Finlay 1982; Decamp 1996). Between 12 and 20 aliquots (5 or 10 L) were pipetted out from each subsample, poured onto a Petri dish and observed with a Nikon inverted microscope at 100×, 200× and 400× total magnifications. Ciliates were classified as free-swimming, crawling or attached (Curds 1973). Occurrence of other microeukaryotes, i.e. free-living amoebae, flagellates and metazoans (rotifers and nematodes), was also noted.

Statistical analyses were carried using SigmaStat for Windows 2.03 (SPSS, San Rafael, CA) and Statistica for Windows 5.1 (StatSoft, Tulsa, OK).

Results

Physico-chemistry and chlorophyll

Temperature was 24.5–29.0 °C in the morning and 28.1–33.4 °C in the afternoon, and the concentration of dissolved oxygen was within the range 5.57–8.30 mg L^{-1} in the morning and 5.27–7.60 mg L^{-1} in the afternoon (Table 1). Dissolved oxygen concentration was significantly higher in tanks with salinity of 9‰ and significantly lower in tanks with salinity of 36‰ (Dunn's test; $P < 0.05$). The pH (Table 1), ranging from 7.3 to 9.25, was significantly higher in tanks with salinity of 9‰ and significantly lower in tanks with salinity of 36‰ (Tukey test; $0.005 < P < 0.001$). For each tank, the concentration of total ammonia remained below 100 μM for the first 20 days of production and below 200 μM for the first 5 weeks of production (Fig. 1). Highest nitrate concentrations were recorded in the first week of production, i.e. 50.9 μM (9‰), 73.0 μM (18‰) and 90.2 μM (36‰). Nitrate remained below 5 μM afterwards. Nitrite concentration during the trial remained below 2.3, 5.5 and 10.1 μM in tanks with salinities of 9‰, 18‰ and 36‰ respectively (Fig. 1). Concentrations of orthophosphate, in the first 31 days of production, remained below 12.7, 16.5 and 18.9 μM in tanks with salinities of 9‰, 18‰ and 36‰ respectively. In the second month of production, orthophosphates reached 73.4, 71.4 and 79.3 μM in tanks with salinities of 9‰, 18‰ and 36‰ respectively (Fig. 1). There were no statistically significant differences between salinity treatments for total ammonia, nitrate, nitrite and orthophosphate measurements.

Concentration of TSS increased with time (Fig. 2) and reached values up to 536, 490 and 989 mg L^{-1} TSS in tanks with salinities of 9‰, 18‰ and 36‰ respectively. The coefficient of determination of the linear regressions ranged from 0.525 to 0.917. In each treatment, Chl *a* linearly increased with time (Fig. 3), reaching up to 1131, 802 and 900 μg L^{-1} Chl *a* in tanks with salinities of 9‰, 18‰ and 36‰ respectively. The coefficient of determination of the linear regressions ranged from 0.511 to 0.928. The slope of the linear regression between TSS or Chl *a* and time did not show a significant difference between treatments.

Ciliates

The number of ciliated protozoa varied strongly with time and treatment, reaching maxima between 4375 (18‰) and 6258 cells mL^{-1} (36‰). Over the whole production cycle, the concentration of ciliates averaged between 1174 (18‰) and 1505 cells mL^{-1} (36‰). An initial peak in abundance of

Table 1 Physicochemistry of tanks with salinities of 36‰, 18‰ and 9‰

Salinity (‰)	pH	Temperature (°C) morning	Temperature (°C) afternoon	DO (mg L^{-1}) morning	DO (mg L^{-1}) afternoon
36	$^{a}8.1 \pm 0.2$	26.89 ± 0.85	30.93 ± 1.25	$^{a}6.63 \pm 0.22$	$^{a}6.02 \pm 0.24$
18	$^{b}8.3 \pm 0.3$	26.94 ± 0.75	31.35 ± 1.05	$^{b}7.06 \pm 0.31$	$^{b}6.29 \pm 0.37$
9	$^{c}8.6 \pm 0.3$	26.52 ± 0.79	30.72 ± 1.04	$^{c}7.41 \pm 0.47$	$^{c}6.61 \pm 0.48$

For each parameter, average ± standard deviation is given. DO values with different superscript letter are significantly different (Dunn's test following Kruskal–Wallis one-way analysis of variance on ranks; $P < 0.05$). Values of pH with different superscript letters are significantly different (Tukey test following one-way ANOVA; $0.005 < P < 0.001$).

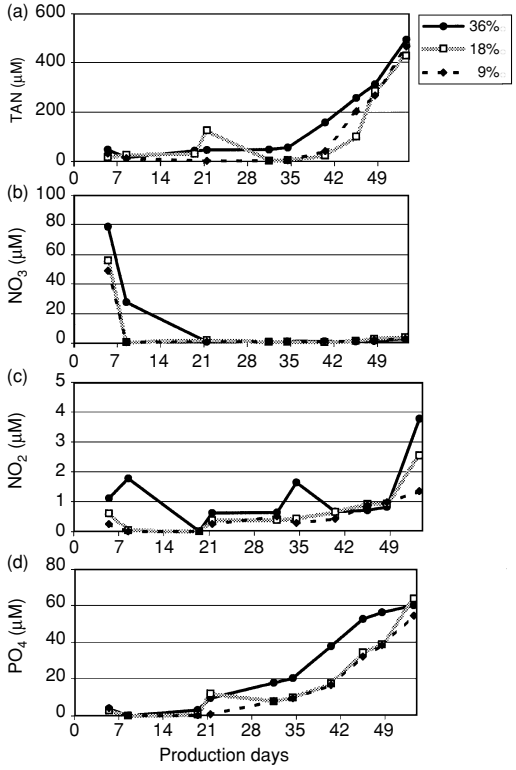

Figure 1 Average concentration of (a) total ammonia (TAN), (b) nitrate (NO_3), (c) nitrite (NO_2) and (d) orthophosphate (PO_4) in tanks with salinities of 9‰, 18‰ and 36‰, over 8 weeks.

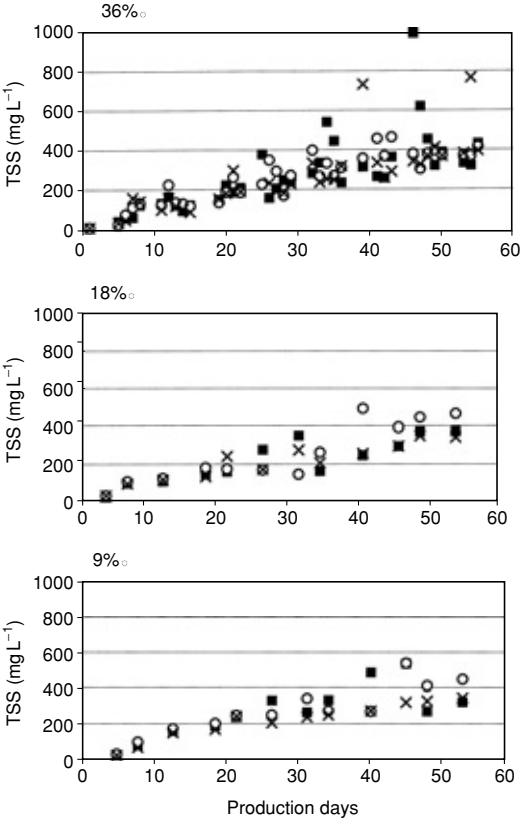

Figure 2 Concentration of total suspended solids (TSS), in tanks with salinities of 9‰ (bottom), 18‰ (middle) and 36‰ (top), over 8 weeks. Points represent the average of two measurements per tank.

ciliates was observed after 1 or 2 weeks of production in all tanks with salinities of 18‰ and 36‰. Ciliates in tanks with a salinity of 9‰ did not show the same trend (Fig. 4). During the first 2 weeks of production, free-swimming ciliates were more abundant in 36‰ than in 9‰ tanks. The highest concentration of free-swimming ciliates (6000 cells mL^{-1}) was observed at highest water salinity, whereas the highest concentration of substrate-associated ciliates (1400 cells mL^{-1}) was observed at 18‰. In each treatment, free-swimming ciliates were dominant in the early stages of production (Table 2). In tanks with salinities of 36‰ and 18‰, the concentration of free-swimming ciliates in the first 2 weeks was significantly higher than that in weeks 3–4 (Dunn's test, $P < 0.05$). Crawling and attached ciliates became more numerous after 2 weeks of production. In the first 4 weeks of production, crawling ciliates were significantly more abundant in 36‰ tanks than in 9‰ tanks (Dunn's test, $P < 0.05$). In 18‰ tanks, the concentration of crawling ciliates was significantly higher in weeks 3–4 than in weeks 1–2 and weeks 5–6 (Dunn's test, $P < 0.05$). In the last 4 weeks of production, the numbers of attached ciliates was significantly higher in 36‰ tanks than in 18‰ tanks (Dunn's test, $P < 0.05$). Finally, in 36‰ tanks, the number of attached ciliates in weeks 3–6 was significantly higher than that in weeks 1–2 (Dunn's test, $P < 0.05$).

Other organisms

Marine rotifers such as *Brachionus plicatilis* and *Lepadella* spp. were observed in all tanks after 12–13 days of production. Bdelloid rotifers were observed in 9‰ tanks after 27 days of production. These rotifers were also observed in 18‰ tanks after 44 days of production. Average concentrations of rotifers were higher in 9‰ tanks (115.1

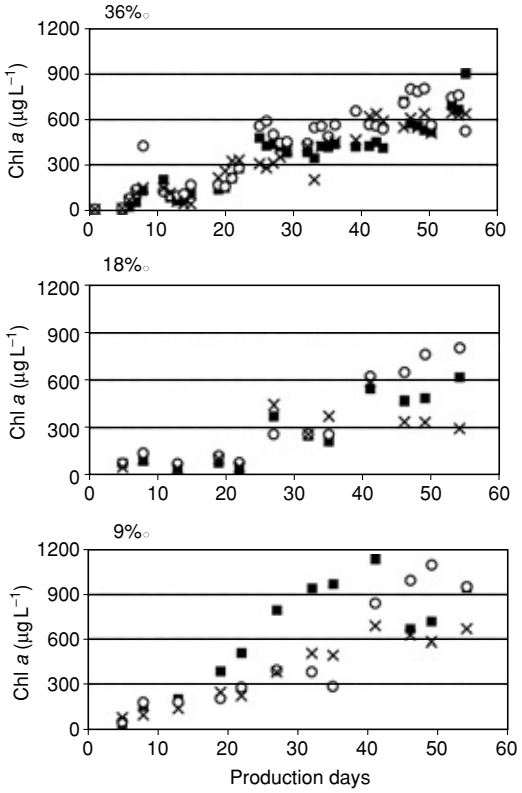

Figure 3 Concentration of chlorophyll *a* (Chl *a*), in tanks with salinities of 9‰ (bottom), 18‰ (middle) and 36‰ (top), over 8 weeks. Points represent the average of two measurements per tank.

ind mL^{-1}) than in 18‰ tanks (44.3 ind mL^{-1}) and 36‰ tanks (14.5 ind mL^{-1}). Nematodes were observed in 36‰ tanks after 32 days of production. Flagellates, sometimes the dominant microeukaryotes, were found to be present. However, their abundance could not be estimated.

Shrimp performance

The final body weight of shrimp reared at 9‰ was significantly lower than that of shrimp reared at 36‰ (Table 3). There was no significant difference between survival rates of shrimp reared at 9‰, 18‰ or 36‰. The final biomass reached 0.35–0.63 kg m^{-2}, equivalent to 0.54–0.97 kg m^{-3}. Treatment influenced the final biomass (Kruskal–Wallis one-way analysis of variance on ranks, $P = 0.029$), but differences between treatments were not statistically significant (Dunn's method).

Discussion

The three treatments were characterized by an identical initial shrimp stocking density and similar feed input (i.e. feed input adapted to shrimp stocking density and therefore influenced by shrimp mortalities). Jiang *et al.* (2000) reported that there were significant effects of temperature and salinity on the excretion rates for ammonia, nitrite and nitrate by *L. vannamei*. We do not have data on the excretion rates of *L. vannamei* in our tanks, but there was no difference between salinity treatment regarding total ammonia, nitrate and nitrite concentrations. Water salinity did not seem to impact nitrogen dynamics significantly within the culture environment, either directly (through the activity of nitrifying bacteria) or indirectly (through nitrogen retention or excretion by shrimp). For each treatment, variations in total ammonia nitrogen, nitrate and nitrite reflects: (i) the accumulation of nitrogen within a zero-water exchange production system, (ii) the increasing concentration of total ammonia nitrogen (Fig. 1a) resulting from protein metabolism and microbial ammonification of organic nitrogen and (iii) the increasing concentration of nitrite (Fig. 1c) due to the activity of ammonium-oxidizing bacteria at later stages of the production cycle. Interestingly, the concentration of nitrate (Fig. 1b) remained low after 2 weeks of production, suggesting low abundance/activity of nitrite-oxidizing bacteria in these culture systems. Nitrite-oxidizing bacteria are slow-growing organisms with doubling time ranging from 12 to 32 h (Ehrich, Behrens, Lebedeva, Ludwig & Bock 1995). Future studies should follow the abundance and the activity of the various groups of bacteria involved in nitrification in zero-water exchange systems.

Differences between treatments in concentration of dissolved oxygen reflect the decreasing solubility of oxygen with increasing water salinity. The pH, however, was significantly higher in tanks with a salinity of 9‰ and significantly lower in tanks with a salinity of 36‰. A higher pH could be explained by higher photosynthetic activity. Unfortunately, data on primary production in the current study are not available. However, data on Chl *a* (Fig. 3) and the amount of Chl *a* per unit weight of suspended matter are available (Fig. 5). Tanks with 9‰ salinity were characterized by a higher pH, but also by a significantly higher concentration of Chl *a* per unit weight of suspended matter (1.93 ± 0.72 µg Chl *a*/mg TSS), than tanks with

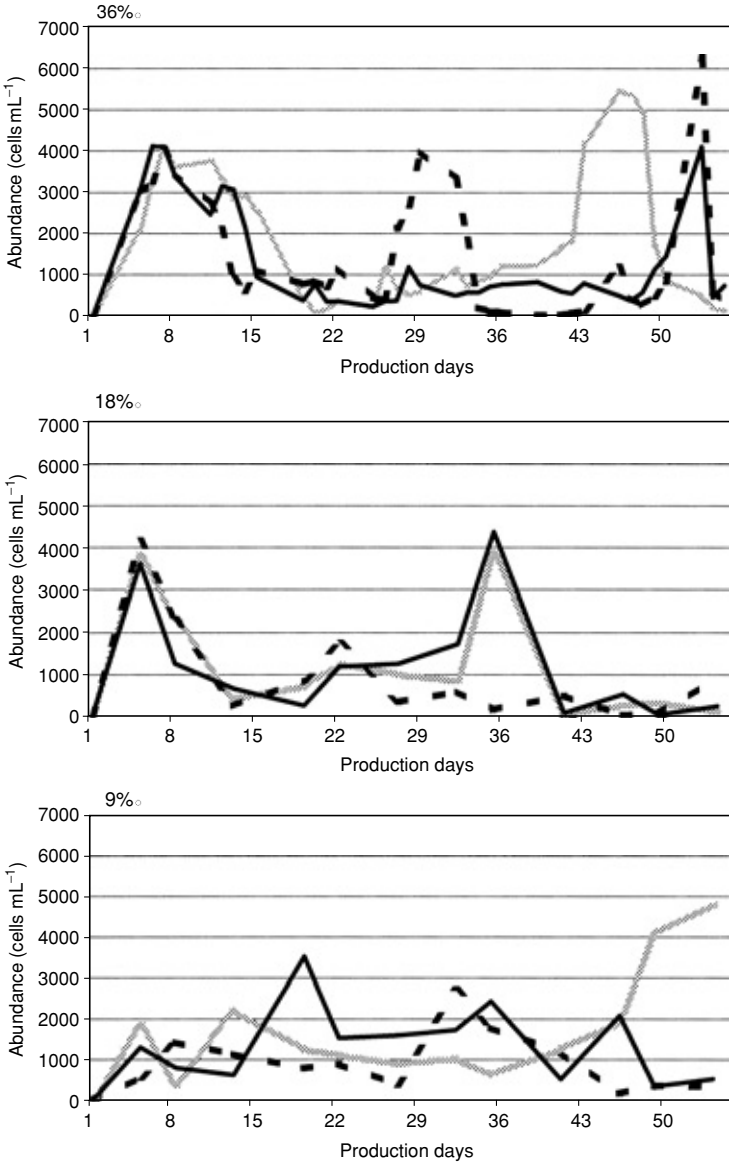

Figure 4 Abundance of ciliated protozoa (cells mL^{-1}) in tanks with salinities of 9‰ (bottom), 18‰ (middle) and 36‰ (top), over 8 weeks. There were three tanks per salinity treatment.

18‰ (1.29 ± 0.68 µg Chl *a*/mg TSS) or 36‰ (1.37 ± 0.61 µg Chl *a*/mg TSS) salinity (Tukey test, $P < 0.05$). Future investigation should provide information on the taxonomical composition and productivity of the phytoplankton encountered at various salinities.

This investigation reaffirms the strong variability existing between replicates of the same treatment, especially for biological parameters. At harvest, Chl *a* concentrations range from around $\pm$ 300 to $\pm$ 800 µg L^{-1} in 18‰ tanks, a ratio of 1 : 3 (Fig. 3). In the 36‰ tanks, there were two peaks in ciliate abundance, whereas another tank showed a third peak in ciliate abundance (Fig. 4), highlighting the difficulty in reproducing results in these experiments.

In the present study, ciliates reached abundances that were higher than those reported in a marine or freshwater planktonic environment (Laybourn-Parry 1992; Leakey, Burkill & Sleigh 1992), but that were within the range of those reported in wastewater treatment (Curds 1982). Concentrations of ciliates showed considerable fluctuations over the sampling period, reflecting the changing physicochemical characteristics of the culture environment, but also the dynamic interactions between

Table 2 Abundance (average ± standard deviation) of free-swimming, crawling and attached ciliates in tanks with salinities of 36, 18 and 9 ppt

	36 ppt	18 ppt	9 ppt	Test
Weeks 1–2				
Free-swimming	2304 ± 1239a	2071 ± 1532ab	1045 ± 621b	Dunn < 0.05
Crawling	186 ± 163a	15 ± 22b	9 ± 14b	Tukey < 0.001
Attached	46 ± 84a	4 ± 11a	90 ± 105a	
Weeks 3–4				
Free-swimming	328 ± 720a	206 ± 117ab	1072 ± 1057b	Tukey < 0.005
Crawling	318 ± 307a	690 ± 450a	28 ± 35b	Dunn < 0.05
Attached	173 ± 163a	70 ± 79a	232 ± 219a	
Weeks 5–6				
Free-swimming	472 ± 896a	1293 ± 1670a	1051 ± 953a	
Crawling	362 ± 344a	34 ± 67b	295 ± 342ab	Dunn < 0.05
Attached	110 ± 101a	29 ± 77b	131 ± 189ab	Tukey < 0.05
Weeks 7–8				
Free-swimming	1259 ± 1789a	110 ± 143b	1476 ± 1787a	Tukey < 0.05
Crawling	277 ± 332a	174 ± 195a	140 ± 196a	
Attached	84 ± 123a	2 ± 4b	10 ± 31b	Dunn < 0.05

Results from one-way ANOVA and Tukey or Dunn a *posteriori* tests on Log $(x+1)$-transformed data are presented. Data on same line with a common letter are not significantly different.

Table 3 Shrimp survival rates (%), feed conversion ratio (FCR), final body weight (g) and final biomass ($kg\,m^{-2}$) in tanks with salinities of 36‰, 18‰ and 9‰

Salinity (‰)	Survival rate (%)	Shrimp FCR	Final body weight (g)	Final biomass ($kg\,m^{-2}$)
36	94.3 ± 1.2	1.68 ± 0.08	^{a}13.40 ± 0.26	0.63
18	93.0 ± 5.3	1.82 ± 0.12	^{ab}12.94 ± 0.21	0.60
9	68.7 ± 50.0	7.95 ± 10.71	^{b}10.23 ± 2.72	0.35

Log-transformed final weights with different superscript letters (a or b) are significantly different (Kruskal–Wallis one-way analysis of variance on ranks; $P < 0.05$).

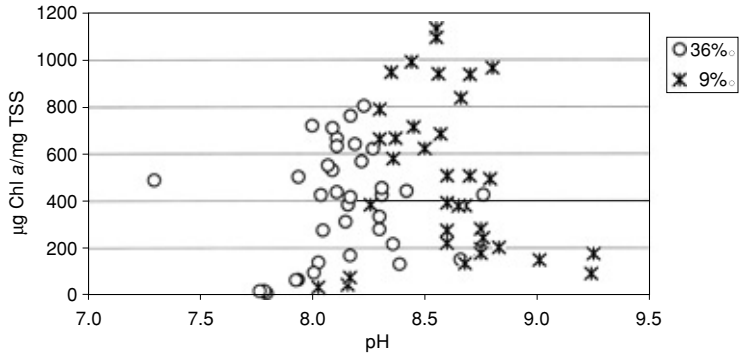

Figure 5 Relationship between water pH and concentration of chlorophyll *a* per dry weight of suspended matter (µg of Chl *a*/mg of TSS) for tanks with salinities of 9‰ and 36‰.

ciliates and their diverse roles within the shrimp production system. The occurrence of various ecological groups of ciliates and abundance of ciliates, in general, was not correlated with measured nutrient concentrations, Chl *a* or TSS. In each tank, the succession of microeukaryotes observed was similar to that reported in activated sludge wastewater treatment plants (Curds 1992; Salvado 1994) or in the colonization process of marine snow (Artolozaga, Santamia, Lopez Ayo & Iriberri 1997). Following the initial peak in flagellates (observed but not measured in the present study), free-swimming ciliates reached a peak within 1–2 weeks of production, and crawling and attached ciliates appeared later. However, clear differences were observed between salinity treatments. Tanks with 36‰ salinity were characterized by: (1) a lack of bdelloid rotifers (phylum Rotifera, class Bdelloidea), (2) a peak of free-swimming ciliates in the first 2 weeks and last 2 weeks of production, and (3) the lowest abundance of attached ciliates in the first 2 weeks of production. Tanks with 18‰ salinity were characterized by: (1) the occurrence of bdelloid rotifers and (2) a peak of free-swimming ciliates in the first 2 weeks of production. Tanks with 9‰ salinity were characterized by: (1) higher abundance of bdelloid rotifers and (2) low initial abundance of free-swimming ciliates. Although bdelloid rotifers are encountered in brackish and marine environments, their species numbers and population densities are generally lower in brackish and, in particular, marine waters than in freshwaters (Örstan 1998).

In a recent investigation of biosecure shrimp production, zooplankton was reported to be dominated by ciliates during the first 20 days of production (Bratvold *et al.* 1999). In the present zero-water exchange production system, ciliates also dominated the zooplankton. Copepods were never observed and rotifers, although present for most of the production cycle, reached lower concentrations than ciliates, i.e. an average of 115 rotifers mL^{-1} with a maximum of 950 rotifers mL^{-1} (tanks with 9‰ salinity), compared with an average of 1505 ciliates mL^{-1} with a maximum of 6258 ciliates mL^{-1} (tanks with 36‰ salinity). However, at certain times, their occurrence and abundance may have impacted the occurrence of ciliates through competition (Laybourn-Parry 1992). Bdelloid rotifers generally grow better on bacteria and yeast (Ricci 1984), and may have to compete with bacterivorous ciliates in low salinity tanks. Nematodes were only observed in 36‰ tanks.

The final shrimp biomass reached 0.54–0.97 kg m^{-3}, or 3.5–6.3 tonnes ha^{-1}, and was within the range of yields given for intensive shrimp production system, i.e. 0.5–10 kg m^{-3} or 5–10 tonnes ha^{-1} (Hopkins, Sandifer & Browdy 1995; Davis & Arnold 1998; Fitzsimmons 1999). As reported by Bray *et al.* (1994), for *L. vannamei* juveniles cultured at salinities of 5–49‰, survival rates did not differ within investigated salinity treatments, i.e. 9–36‰. The final body weight of shrimp reared at a salinity of 36‰ was higher than that of shrimp reared at a salinity of 9‰, and the feed conversion ratio (FCR) of shrimp reared at a salinity of 9‰ was higher than that of shrimp reared at the other two salinities – although this could be explained by the poor performance of one specific tank (Table 3). This poor result for shrimp reared at a salinity of 9‰ was unexpected because *L. vannamei* are euryhaline: (1) they tolerate salinities of about 2‰ (Moya *et al.* 1999), (2) they grow well at low salinity, with little difference in growth rates noted among salinities of 20–50‰ (Ponce-Palafox *et al.* 1997) and (3) maximum growth rate was obtained in shrimp maintained at 15‰ salinity (Rosas, Cuzon, Gaxiola, Priol, Pascual, Rossignyol, Contreras, Sanchez & Wormhoudt 2001). The lower growth rate of shrimp reared in a salinity of 9‰ could be related to the mineral composition of the water. Low-salinity growth trials undertaken by Scarpa & Vaughan (1998) and Allen, Laramore, Fung, Duerr & Scarpa (2000) found that a high chlorine concentration (300 mg L^{-1}) and a hardness of $\geq$ 150 mg L^{-1} $CaCO_3$ may be necessary for successful low salinity culture of *L. vannamei*. Another possible reason for the lower growth rate of shrimp reared in a salinity of 9‰ could be an acclimatization period that was too short. In the current experiment, shrimp were acclimated down from 36‰ by reducing the salinity by 50% every 12 h. However, these shrimp were then maintained at the desired salinity for at least 2 weeks before trial stocking. The combination of low salinity and low crude protein content of the formulated diet may also have led to lower shrimp production. In low salinity, shrimp may use protein as a source of amino acids to maintain the osmotic pressure and for growth (Claybrook 1983 in Rosas *et al.* 2001). Indeed, both Rosas *et al.* (2001) and Shiau (1998) reported that shrimp reared at salinities of 15–16‰ required a higher protein diet to achieve good growth, i.e. 50% crude protein (CP). Future experiments should be carried out using high-protein formulated diets.

The lower FCR of shrimp reared at lower salinity levels would imply a larger amount of uneaten feed in the zero-water exchange system, and therefore higher total ammonia concentration and a potentially higher concentration of bacterivorous ciliates predating upon the larger number of bacteria associated with the detritus and increased nutrients. However, there were no significant differences in total ammonia or nitrite concentrations (Fig. 1) – in fact, nitrite concentration tended to be lower in the 9‰ tanks – and in ciliate abundance (Fig. 4; Table 2).

Although the poor performance of shrimp reared at 9‰ suggests that the experimental protocol may not have been optimized (i.e. mineral composition of the water, length of acclimatization period and crude protein content of the formulated diet), results indicate that water salinity did not influence water nutrients, but rather, within this specific zero-water exchange production system, had an impact on (1) water pH, (2) concentration of Chl *a* per weight of suspended matter and (3) abundance, occurrence and succession of various ecological groups of ciliates. Regarding this latter point, parallels between this zero-water exchange shrimp production system and wastewater environment (i.e. high organic load, high abundance of protozoa and biochemical composition of the flocculated matter (Decamp, Conquest, Forster & Tacon 2002) indicate that the dynamics of protists could be used as an indicator of overall water quality or performance of shrimp production systems. Additionally, the contribution of ciliates as a food source should be investigated (Nagano, Iwatsuki, Kamiyama & Nakata 2000). Using a published conversion factor (Putt & Stoeker 1989), four thousand ciliates per mL (Fig. 4) with a doubling time of 12 h would represent a daily production of around 1 g carbon per culture tank, i.e. a relatively small amount compared with the amount of feed added to the system. However, *L. vannamei* has a limited ability to bioconvert fatty acids to polyenoic forms of longer chain length (Lim, Ako, Brown & Hahn 1997). Flagellates and ciliates are a source of polyunsaturated fatty acids in the microbial loop (Zhukova & Kharlamenko 1999), and ciliates, such as the free-swimming ciliates observed in the present study, are reported to be rich in highly unsaturated fatty acids (i.e. 16–25% of the total) (Sul, Erwin, Kaneshiro & Jayasimhulu 2000), which have a good growth-promoting effect on juvenile *L. vannamei* (Lim *et al.* 1997). A formulated feed with 8.9% crude lipids, such as that used in the present study, should provide a reasonable amount of unsaturated fatty acids to the reared animals. In this case, the contribution of ciliates and other organisms present in the culture system to the overall intake of unsaturated fatty acid might be limited. However, we suggest that the biochemical composition of dominant ciliates and their ingestion/assimilation rates by *L. vannamei* should be investigated in order to better understand the trophic interactions within intensive shrimp production system and to improve the formulation of artificial diets.

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References

Allen S.E., Laramore R., Fung J., Duerr L. & Scarpa J. (2000) Low salinity and environmental ionic composition effects on growth and survival of *Litopenaeus vannamei*. *Aquaculture America* (Abstract 4).

Artolozaga I., Santamia E., Lopez A., Ayo B. & Iriberri J. (1997) Succession of bacterivorous protists on laboratory-made marine snow. *Journal of Plankton Research* **19**, 1429–1440.

Bratvold D., Lu J. & Browdy C.L. (1999) Disinfection, microbial community establishment and shrimp production in a prototype biosecure pond. *Journal of the World Aquaculture Society* **30**, 422–432.

Bray W.A., Lawrence A.L. & Leung-Trujillo J.R. (1994) The effect of salinity on growth and survival of *Penaeus vannamei*, with observations on the interaction of IHHN virus and salinity. *Aquaculture* **122**, 133–146.

Brown J.J. & Glenn E.P. (1999) Reuse of highly saline aquaculture effluent to irrigate a potential forage halophyte, *Suaeda esteroa*. *Aquaculture Engineering* **20**, 91–111.

Brown J.J., Glenn E.P., Fitzsimmons K.M. & Smith S.E. (1999) Halophytes for the treatment of saline aquaculture effluent. *Aquaculture* **175**, 255–268.

Chen J.-C. & Lin C.-Y. (1995) Responses of oxygen consumption, Ammonia-N excretion and Urea-N excretion of *Penaeus chinensis* exposed to ambient ammonia at different salinity and pH levels. *Aquaculture* **136**, 243–255.

Curds C.R. (1973) The role of ciliates in the activated-sludge process. *American Zoology* **13**, 161–169.

Curds C.R. (1982) The ecology and role of protozoa in aerobic sewage treatment processes. *Annals of Reviews in Microbiology* **36**, 27–46.

Curds C.R. (1992) *Protozoa and the Water Industry*. Cambridge University Press, Cambridge, UK.

Davis D.A. & Arnold C.R. (1998) The design, management and production of a recirculating raceway system for the production of marine shrimp. *Aquaculture Engineering* **17**, 193–211.

Decamp O. (1996) *The microbial ecology of the root zone method of wastewater treatment*. PhD Thesis. University of Leicester, Leicester, UK.

Decamp O., Conquest L., Forster I. & Tacon A.G.J. (2002) The nutrition and feeding of marine shrimp within zero-water exchange aquaculture production systems: role of eukaryotic microorganisms. In: *Microbial Approaches to Aquatic Nutrition Within Environmentally Sound Aquaculture Production Systems* (ed. C.-S. Lee & P.O'Bryen), pp. 79–86. The World Aquaculture Society, Baton Rouge, FL, USA.

Dinçer A.R. & Kargi F. (2001) Salt inhibition kinetics in nitrification of synthetic saline wastewater. *Enzyme Microbiology and Technology* **28**, 661–665.

Ehrich S., Behrens D., Lebedeva E., Ludwig W. & Bock E. (1995) A new obligately chemolithoautotrophic, nitrite-oxidizing bacterium, *Nitrospira moscoviensis* sp. nov. and its phylogenetic relationship. *Archives of Microbiology* **164**, 16–23.

Finlay B.J. (1982) Procedures for the isolation, cultivation and identification of protozoa. In: *Experimental Microbial Ecology* (ed. R.G. Burns & J.H. Slater), pp. 44–65. Blackwell Scientific Publications, Oxford.

Fitzsimmons K. (1999) Shrimp farming in saline groundwater in Arizona, USA. *World Aquaculture '99, Sydney, Australia.* World Aquaculture Society (Abstract 263).

Foissner W. (1988) Taxonomic and nomenclatural revision of Sladecek's list of ciliates (Protozoa: Ciliophora) as indicators of water quality. *Hydrobiologia* **166**, 1–64.

Frelier P.F., Loy J.K., Varner P., Thompson J.A., Lawrence A.L. & Bray W.A. (1995) Management procedures for the treatment of necrotizing hepatopancreatitis in farmed shrimp. In: *Swimming Through Troubled Waters, Proceedings of the Special Session on Shrimp Farming* (ed. by C. L. Browdy & J. S. Hopkins), pp. 240. World Aquaculture Society '95. San Diego, CA.

Fukami K., Watanabe A., Fujita S., Yamaoka K. & Nishijima T. (1999) Predation on naked protozoan microzooplankton by fish larvae. *Marine Ecology Progress Series* **185**, 285–291.

Hopkins J.S., Sandifer P.A. & Browdy C.L. (1995) Effect of two protein levels and feed rate combinations on water quality and production of intensive shrimp ponds operated without water exchange. *Journal of the World Aquaculture Society* **26**, 93–97.

Intrasungkha N., Keller J. & Blackall L.L. (1999) Biological nutrient removal efficiency in treatment of saline wastewater. *Water Science and Technology* **39**, 183–190.

Jiang D.-H., Lawrence A.L., Neill W.H. & Gong H. (2000) Effects of temperature and salinity on nitrogenous excretion by *Litopenaeus vannamei* juveniles. *Journal of Experimental Marine Biology and Ecology* **253**, 193–209.

Lachat (2000) *Quikchem Method for Lachat Instruments*. ZellWeger Analytics, Milwaukee, WI, USA.

Laybourn-Parry J. (1992) *Protozoan Plankton Ecology*. Chapman & Hall, London.

Leakey R.J.G., Burkill P.H. & Sleigh M.A. (1992) Planktonic ciliates in Southampton Water: abundance, biomass, production, and role in pelagic carbon floc. *Marine Biology* **114**, 67–83.

Leber K.M. & Pruder G.D. (1988) Using experimental microcosms in shrimp research: The growth-enhancing effect of shrimp pond water. *Journal of the World Aquaculture Society* **19**, 197–203.

Lim C., Ako H., Brown C.L. & Hahn K. (1997) Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. *Aquaculture* **151**, 143–153.

Moss S.M. & Pruder G.D. (1995) Characterization of organic particles associated with rapid growth in juvenile white shrimp, *Penaeus vannamei* Boone, reared under intensive culture conditions. *Journal of Experimental Marine Biology and Ecology* **187**, 175–191.

Moya M., Lawrence A.L., Collins C.A. & Samocha T.M. (1999) Acclimation of *Penaeus vannamei* postlarvae to 2 ppt ground saline water in Sonora Desert, Arizona. *World Aquaculture '99, Sydney, Australia.* World Aquaculture Society (Abstract 424).

Nagano N., Iwatsuki Y., Kamiyama T. & Nakata H. (2000) Effects of marine ciliates on survivability of the first-feeding larval surgeonfish, *Paracanthurus hepatus*: laboratory rearing experiments. *Hydrobiologia* **432**, 149–157.

Örstan A. (1998) Microhabitats and dispersal routes of bdelloid rotifers. *Scientiae Naturae* **1**, 27–36.

Ponce-Palafox J.T., Martinez-Palacios C.A. & Ross L.G. (1997) The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. *Aquaculture* **157**, 105–113.

Putt M. & Stoeker D.K. (1989) An experimentally determined carbon: volume ratio for marine oligotrichous ciliates from estuarine and coastal waters. *Limnology and Oceanography* **34**, 1097–1103.

Ricci C. (1984) Culturing of some bdelloid rotifers. *Hydrobiologia* **112**, 45–51.

Rosas C., Cuzon G., Gaxiola G., Priol Y.L., Pascual C., Rossignyol J., Contreras F., Sanchez A. & Wormhoudt A.V. (2001) Metabolism and growth of juveniles of *Litopenaeus vannamei*: effect of salinity and dietary carbohydrate levels. *Journal of Experimental Marine Biology and Ecology* **259**, 1–22.

Salvado H. (1994) Effect of mean cellular retention time on ciliated protozoan populations in urban wastewater treatment plants based on a proposed model. *Water Research* **28**, 1315–1321.

Scarpa J. & Vaughan D.E. (1998) Culture of the marine shrimp, *Penaeus vannamei*, in freshwater. *Aquaculture '98*. World Aquaculture Society (Abstract 473).

Shiau S.-Y. (1998) Nutrient requirements of penaeid shrimps. *Aquaculture* **164**, 77–93.

Stoeker D.K. & Capuzzo J.M. (1990) Predation on protozoa: its importance to zooplankton. *Journal of Plankton Research* **12**, 891–908.

Strickland J.D.F. & Parsons T.R. (1972) A practical handbook of seawater analysis. *Bulletin of the Fisheries Research Board, Canada* **167**, 311 pp.

Sturmer L.N. & Lawrence A.L. (1989) Salinity effects on *Penaeus vannamei* production in nursery and growout ponds. *Journal of the World Aquaculture Society* **20** (Abstract 73A).

Sul D., Erwin J.A., Kaneshiro E.S. & Jayasimhulu K. (2000) Neutral lipids, their fatty acids, and the sterols of the marine ciliated protozoon, *Parauronema acutum*. *Journal of Eukaryotic Microbiology* **47**, 373–378.

Zhukova N.V. & Kharlamenko V.I. (1999) Sources of essential fatty acids in the marine microbial loop. *Aquatic Microbial Ecology* **186**, 199–210.