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EFFECT OF TWO STOCKING DENSITIES ON THE GROWTH AND MORTALITY OF THE PINK ABALONE *HALIOTIS CORRUGATA* IN RECIRCULATING AND FLOW-THROUGH SYSTEMS

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ABSTRACT Traditional abalone culture is carried out in flow-through systems with water exchange rates between 200 and 2,400% of the total tank volume per day. These high volumes of water associated with abalone culture represents a constraint for the growth of this industry, and recirculating systems can become a viable alternative, because the water exchange rates are less than 10% of the total volume. The objective of this experiment was to evaluate the water quality parameters and the growth rate and mortality of the pink abalone cultured at two different densities, 10% (D1) and 30% (D2) in a flow-through system (S1) and two closed recirculating systems (S2 and S3). A total of 2,400 juvenile abalone (7.11 ± 2.0 g and 37.01 ± 3.4 mm) were distributed among the three experimental systems. The experiment lasted for 127 days. Weight and length of all the abalone were measured at the beginning and end of the experiment. Average water quality parameters for each system (S1, S2, and S3) were respectively: temperature (18.2 ± 1.4, 18.4 ± 1.9, and 18.1 ± 2.0°C); salinity (33.8 ± 1.0, 34.5 ± 1.2, and 34.2 ± 1.5‰); alkalinity (127 ± 13.2, 135 ± 13.7 and 131 ± 14.1 mg of $CaCO_3$/L), total ammonia nitrogen (0.006 ± 0.02, 0.03 ± 0.07, and 0.02 ± 0.08 mg TAN/L) and nitrite (0 ± 0.00, 0.08 ± 0.07, and 0.07 ± 0.05 mg NO_2/L). Growth rates in weight (g/d) were for S1D1 (0.027 ± 0.007), S1D2 (0.018 ± 0.001), S2D1 (0.007 ± 0.001), S2D3 (0.005 ± 0.000), S3D1 (0.007 ± 0.000) and S3S2 (0.009 ± 0.003), and growth rates in length (mm/d) were for S1D1 (0.038 ± 0.004), S1D2 (0.031 ± 0.002), S2D1 (0.013 ± 0.001), S2D3 (0.013 ± 0.000), S3D1 (0.002 ± 0.000), and S3D2 (0.024 ± 0.001) respectively. In flow through systems growth in length and width was density dependent. Mortality was higher in S3D2 than in any other treatment. A lower water exchange rate in closed systems could adversely affect growth and mortality. Abalone culture in closed recirculating systems could become another alternative for this industry, but more research is required.

KEY WORDS: abalone, *Haliotis corrugata*, recirculating system, growth, mortality

INTRODUCTION

Abalone (*Haliotis* spp.) fishery has been one of the most important in the northwest part of Mexico over the past 40 y. Several factors such as overfishing and diseases have had a toll on landings, which have decreased dramatically from 3,000 mt in the 1970s, to less than 300 mt nowadays (Salas-Garza & Searcy-Bernal 1992, Anonymous 2000). This drastic reduction in abalone capture increased the interest in their aquaculture. Abalone culture officially began in Mexico in the 1990s, and currently all farms are land-based. In these farms, abalone culture is characterized by high water flow rates to keep optimum water quality parameters within levels recommended for grow-out conditions. The rate of water exchange in an abalone culture tank normally ranges from 200% to 2,400% per day. The cost associated with the maintenance of this high rate of exchange accounts for between 15% to 30% of the production costs in an abalone farm. One way to decrease the cost of production associated with constantly pumping water through the abalone grow-out tanks, can be by the use of recirculation technology. Recirculating systems have been successfully used elsewhere to grow tilapia, turbot, artic char, and northern quahog among others species (Pfeiffer et al. 1999, Timmons et al. 2002, Labatut & Olivares 2004, Summerfelt et al. 2004). Biofiltration, solids removal, circulation, aeration, and degasification are the five most important characteristics for the efficient operation of any recirculating system. In abalone culture, recirculation has been used for broodstock maintenance and conditioning, and grow-out experiments with species such as *H. discus hannai* and *H. tuberculata* (Park et al. 1995, Nie et al. 1996, Mgaya & Mercer 1995, Sawa 2000). *Haliotis discus hannai* has also been cocultured in recirculating systems with the red algae *Palmaria mollis* with the benefit of maximizing biofilter function and reducing the amount of nutrients to the effluents (Demetropoulos & Langdon 2004). So far, no studies have been conducted with *H. corrugata* in recirculating systems. The results obtained from this experiment might set the road for the transformation of the way abalone is cultured in México.

MATERIALS AND METHODS

Abalone Stock and Culture Systems

Juvenile pink abalone *Haliotis corrugata* were obtained from B.C. Abalone and Productores Marinos Baja (2,400, 7.2 ± 2.0 g, 37.01 ± 3.4 mm), Ejido Eréndira, Baja California, México. At their arrival to CICESE, Aquaculture Department, the abalone were placed in plastic cages in a flow-through system, and the juvenile abalone were acclimated for 42 days at 20°C. During this period, abalone were fed *ad libitum* with macroalgae *Macrocystis pyrifera* and exposed to natural photoperiod.

One flow-through system and two recirculating systems were used in the growth trial. The flow-through system (S1) consisted of six black round flat bottom tanks (280-L capacity). The water used in this experiment was pumped from the ocean, filtered to 70 μm and UV-irradiated before reaching the tanks. A flow rate of 0.5 L/min was provided to each one of the six tanks for a daily

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rate of water exchange of 290%. Each of the two recirculating systems used in this experiment (S2 and S3) consisted of three round flat bottom tanks (280 L capacity) connected to a 180 L sump. A bubble-washed, bead filter (0.056 m^3 polyethylene beads) was used to provide biofiltration and clarification to each system. Both bead filters were acclimated for 48 days at 20°C prior to the experiment as recommended by Simonel et al. (2002). In each system, water was recirculated with a 1/6 HP centrifugal pump. Flow rate to each tank was 3 L/min. For the flow-through and the recirculating system, air was provided by air-stones connected to an electric blower. Water temperature was maintained using 250 W titanium heaters for each tank in the flow-through system, and 800 W heaters in each of the two recirculating system.

In each tank, four plastic cages (0.25 m × 0.25 m × 0.3 m, 0.012 m square mesh size) were placed, and within each cage, two polyvinyl chloride gutters (0.24 m × 0.26 m) were used to provide for available area for the abalone (0.1248 m^2). All abalones used in the experiment were fed with *M. pyrifera* at a rate of 15% body weight/day and leftover macroalgae were regularly removed from the tanks. During the experiment, temperature and salinity were measured daily (conventional thermometer and refractometer). Total ammonia-nitrogen (TAN), nitrate, and alkalinity were measured twice a week (HACH FF3 tests). Alkalinity was maintained in both recirculating systems by placing abalone shells in the sump as recommended for volatile, low-density systems (Malone & Burden 1988, Malone et al. 1996, Malone & Beecher 2000, Timmons et al. 2002).

The experiment was conducted from December 2004 to March 2005 and data were averaged for each month.

Experimental Design

Two stocking densities were evaluated in the flow-through and recirculating systems. The densities used in this experiment were 10% (D1) and 30% (D2) of available surface area, 26 and 74 abalone per cage (104 and 297 abalone per tank). In S1, a total of 6 tanks (3 tanks-D1, and 3 tanks-D2), were used. For S2 and S3, both densities were distributed as follows: S2, two tanks D1, and one tank D2, and S3, one tank D1, and two tanks D2. Because of logistic problems, densities could not be equally replicated in these recirculating systems. All abalone were dislodged manually in each cage, blot dried, weighted to the nearest 0.1 g body weight, and shell length and width was measured to 0.1 mm with a digital caliper at the beginning of the experiment (day 0) and at the end of the experiment (day 127). At the time of feeding, mortality was removed from each cage and quantified.

Abalone growth rate (AGR) (growth rate.day^{-1}) was calculated for body weight, shell length, and width by the following equation:

$$Growth\ rate = \frac{(final\ -\ initial)}{time\ (days)}$$

Statistical Analyses

After all data were analyzed for normality and homogeneity, all water quality parameters and mortality were analyzed with a one-way ANOVA to determine if significant differences existed among S1, S2, and S3 in the different months evaluated. When significant differences were indicated, treatment means were separated with a Tukey HSD test at a level of significance of 0.05.

A two-factor ANOVA was used to detect differences among systems (S1, S2, and S3) and densities (D1 and D2) in growth rates in total weight, shell length, and width When significant differences were indicated, orthogonal contrasts tests were conducted between treatment means at a level of significance of 0.05 (SAS 1985).

RESULTS

Water Quality

The salinity means (±SD) throughout the experiment for S1, S2, and S3 were 33.76 ± 0.97‰, 34.47 ± 1.2‰, and 34.24 ± 1.53‰ respectively. Significant differences in salinity were detected in January between S1 and S2, and S3 was similar to both ($F = 4.76$, $P = 0.0219$). No significant differences were detected in the other months (Fig. 1). Temperature means (±SD) were 18.2 ± 1.4, 18.4 ± 1.9, and 18.1 ± 2.0°C, for S1, S2, and S3, respectively. A power outage at the 13th day of experiment and a malfunction in the heaters at the 30th day caused a temperature fluctuation in the first month of the growth trial in both recirculating systems (22.6°C to 13.1°C in S2, and 23°C to 13.5°C in S3). Significant differences in temperature were detected in February where S2 was different from S1 and S3 ($F = 5.85$, $P = 0.0096$). In March, S1 was significantly different from S3, and S2 was similar to both ($F = 4.60$, $P = 0.0166$) (Fig. 2). Ammonia means (±SD) for S1, S2, and S3 respectively, were 0.006 ± 0.02, 0.03 ± 0.07, and 0.02 ± 0.08 mg TAN/L. No significant differences in TAN were detected among the three systems throughout the experiment (Fig. 3). However, in the first month (December) the closed systems showed higher concentrations of TAN (up to 0.3 mg/L). Nitrite means (±SD) were 0 ± 0.00, 0.08 ± 0.07, and 0.07 ± 0.05 mg NO_2/L, for S1, S2, and S3, respectively. Significant

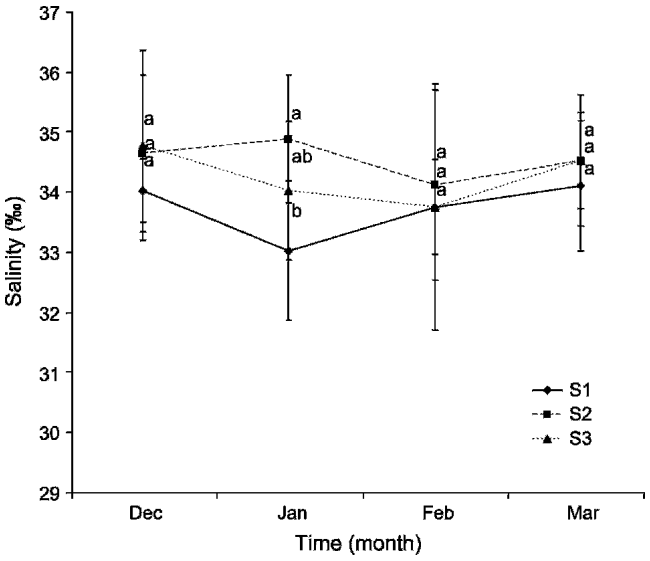

Figure 1. Salinity values (‰) obtained (mean ± SD) over the duration of the experiment. In each month, points on different lines with the same letter are not significantly different ($P > 0.05$).

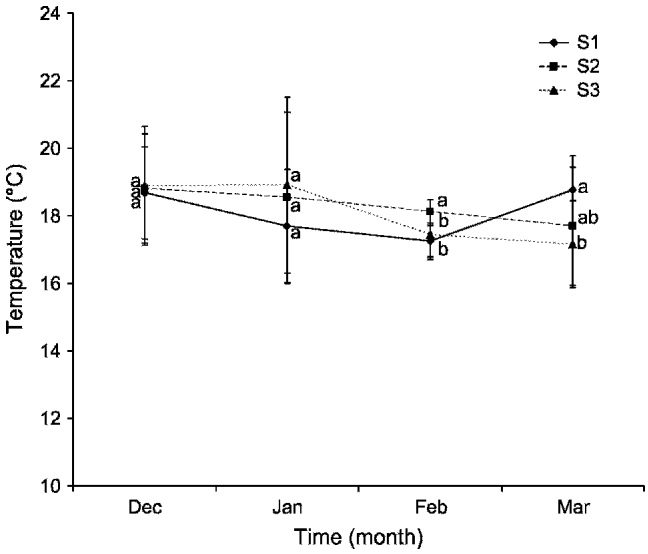

Figure 2. Temperature values (°C) obtained (mean ± SD) over the duration of the experiment. In each month, points on different lines with the same letter are not significantly different ($P > 0.05$).

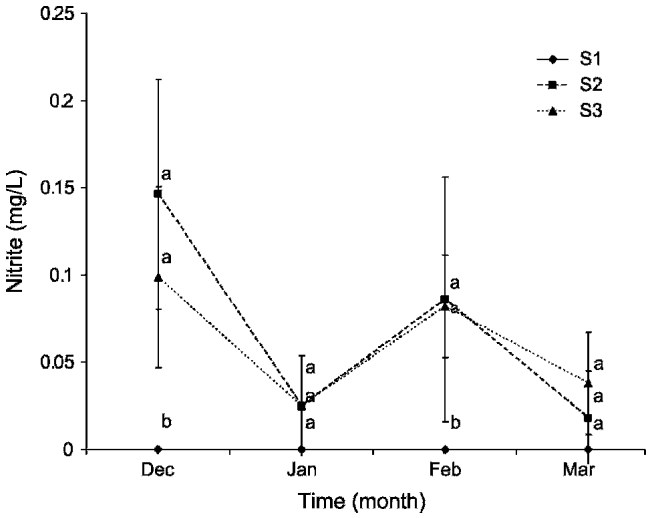

Figure 4. Nitrite values (mg/L) obtained (mean ± SD) over the duration of the experiment. In each month, points on different lines with the same letter are not significantly different ($P > 0.05$).

differences in nitrites were detected in December, where S1 was significantly different from S2 and S3 ($F = 19.05$, $P = 0.0001$). Significant differences were also detected in February where S1 was significantly different from S2 and S3 ($F = 6.09$, $P = 0.0149$) (Fig. 4). Alkalinity means (±SD) were 127 ± 13.2, 135 ± 13.7, and 131 ± 14.1 mg of $CaCO_3$/L, for S1, S2, and S3 respectively. No significant differences in alkalinity were detected between the three systems in all the culture periods (Fig. 5). A volume of 720 L/day of water was required for each tank in S1, 91,440 L per tank for the whole experiment, and a total of 548,640 L for S1 for all six tanks. Each of the closed systems used a total volume of 6,375 L in for the experiment, including 430 L of freshwater used to replenish water lost by evaporation. Water exchange rates are shown in Table 1.

Abalone Growth and Mortality

The two-way ANOVA in growth rate in weight (GRW) was significant among systems ($F = 19.31$, $P = 0.0024$) but no differences caused by densities or interactions were detected ($F = 1.57$, $P = 0.2564$ and $F = 1.30$, $P = 0.3395$, respectively). The contrasts tests showed that weight in the open system (S1) was significantly higher than in the closed systems (S2 and S3) ($F = 38.31$, $P = 0.0008$) (Fig. 6).

Growth rate in shell length (GRL) was significantly different among systems ($F = 45.27$, $P = 0.0002$) but not between densities ($F = 1.44$, $P = 0.2753$), although a significant interaction was also detected ($F = 8.12$, $P = 0.0196$). The contrasts tests showed that GRL was significantly higher in S1 than in S2-S3 ($F = 89.61$, $P = 0.0001$), but did not differ between S2 and S3 ($F = 0.94$, $P = 0.3705$). The significant interaction may be explained by the lower GRL for D2

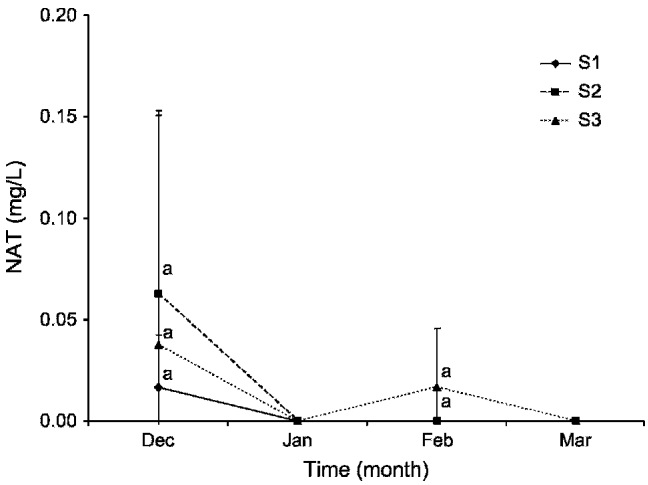

Figure 3. Total ammonia nitrogen values (mg/L) obtained (mean ± SD) over the duration of the experiment. In each month, points on different lines with the same letter are not significantly different ($P > 0.05$).

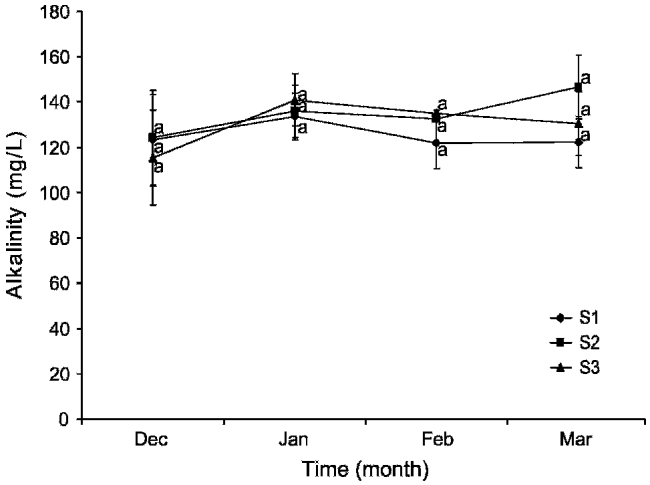

Figure 5. Alkalinity values (as mg/L of $CaCO_3$) obtained (mean ± SD) over the duration of the experiment. In each month, points on different lines with the same letter are not significantly different ($P > 0.05$).

TABLE 1.

Average water exchange rate per day throughout the experiment in the three systems (S1, S2, and S3).

	Time (days)			
	December	January	February	March
S1	289%	289%	289%	289%
S2	4.92%	2.66%	2.41%	1.98%
S3	4.92%	2.66%	2.41%	1.98%

compared with D1 in S1, contrasting with the higher values in the closed systems, especially in S3 (Fig. 7).

Growth rate in shell width (GRWd) was significantly different among systems ($F = 39.51$, $P = 0.0004$), but no densities or interaction effects were detected ($F = 4.39$, $P = 0.0810$ and $F = 2.78$, $P = 0.1399$, respectively). The contrast tests showed that shell width in the open system was significantly higher in S1 than in S2 and S3 ($F = 78.49$, $P = 0.0001$) (Fig. 8).

The total mortality rate during the growth trial was 16.67% (400 abalone).The mortality rates for D1 and D2, were 15.22% (95 abalone) and 17.17% (305 abalone), respectively. The mortality rates for S1, S2, and S3 were 10.25% (123 abalone), 15.87% (80 abalone) and 28.3% (197 abalone), respectively. The highest mortality rates were observed in the first month of the experiment, 70, 34, and 118 abalones for S1, S2, and S3; which were equivalent to 56.9%, 42.5%, and 59.8% of the total mortality. In the analysis of mortality for treatment combinations, S3D2 was significantly higher ($F = 13.92$, $P = 0.0001$) from the other treatments (Table 2).

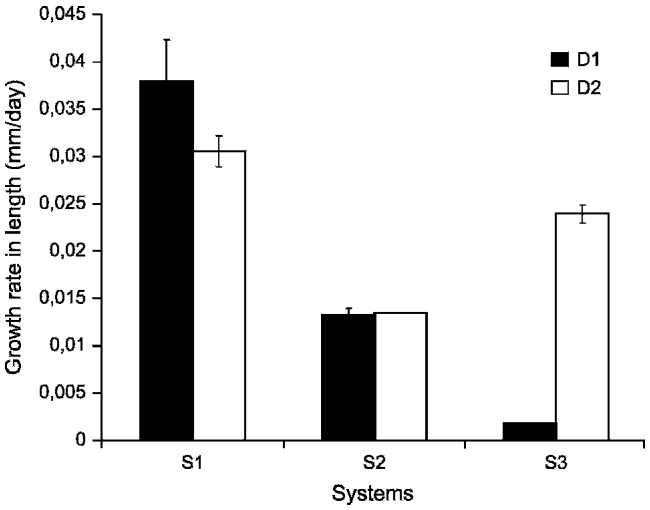

Figure 7. Pink abalone *H. corrugata* growth rate (mean ± SD) in shell length (mm/day) obtained at two different densities (D1 = 10% and D2 = 30%) in a flow-through system (S1) and two recirculating systems (S2 and S3). In each month, points on different lines with the same letter are not significantly different ($P > 0.05$).

DISCUSSION

Recommended concentrations of salinity varies for different abalone species for example as follows: *H. discus hannai* from 25–44‰, *H. rufescens*, 32‰ (± 0.02), *H. tuberculata* 34‰ (±1), *H. laevigata* 34‰, *H. diversicolor supertexta* and *H. fulgens*, 35‰, *H. varia* 32‰ (±2), and *H. asinina* from 30‰ to 34‰

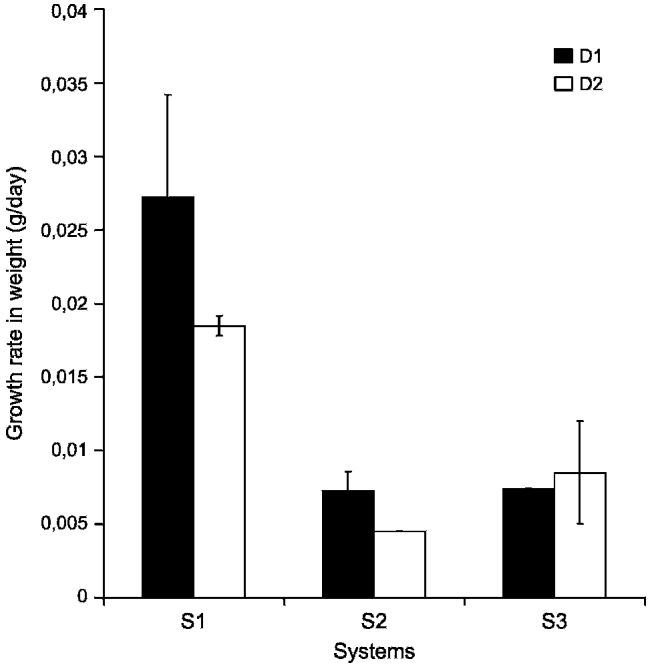

Figure 6. Pink abalone *H. corrugata* growth rate (mean ± SD) in weight (g/day) obtained at two different densities (D1 = 10% and D2 = 30%) in a flow-through system (S1) and two recirculating systems (S2 and S3). In each month, points on different lines with the same letter are not significantly different ($P > 0.05$).

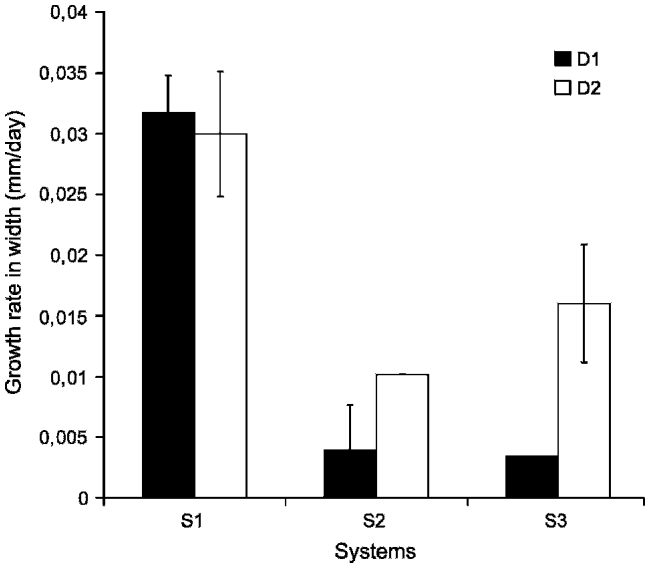

Figure 8. Pink abalone *H. corrugata* growth rate (mean ± SD) in shell width (mm/day) obtained at two different densities (D1 = 10% and D2 = 30%) in a flow-through system (S1) and two recirculating systems (S2 and S3). In each month, points on different lines with the same letter are not significantly different ($P > 0.05$).

TABLE 2.

Pink abalone *H. corrugata* total mortality rate by system and density. Values with the same letter are not significantly different ($P > 0.05$).

System and Density	Stocking Density (% Surface area)	Total Mortality (Number of abalone)	Total Mortality (%)
S1D1	10	33	10.58[b]
S1D2	30	90	10.14[b]
S2D1	10	41	19.71[b]
S2D2	30	39	13.18[b]
S3D1	10	21	20.19[b]
S3D2	30	176	29.73[a]
Total		400	16.67

(Hahn 1989, Basuyaux & Mathieu 1999, Harris et al. 1999, Capinpin et al. 1999, Medina-Romo 2002, Steinarsson & Imsland 2003, Cheng et al. 2004, Najmudeen & Victor 2004). For pink abalone, the recommended salinity is 35‰ (ReAraujo 2003) and in our experiment, salinity ranged between 32.37‰ to 36.72‰. In January, we experienced an unusual amount of rain, the water we used for the flow-through system was directly pumped from the shallow ocean waters and the runoff probably affected salinity.

The temperature range reported for the optimal growth of *H. corrugata* is between 15°C and 23°C (Spencer 2002). Re-Araujo (2003) reported that the preferred temperature for *H. corrugata* was 24.5°C.

Gastropod excretion is mostly composed of nitrogenous compounds, largely ammonia, whereas feces constitute only a marginal source of ammonia (Kinne 1976, Spotte 1979). Ammonia is a toxic and a stressor in aquaculture (Harris et al. 1998, Basuyaux & Mathieu, 1999). Abalone tolerance to TAN varies among the different species, *H. tuberculata* shows toxicity symptoms when TAN is ≥10 mg/L and the recommended concentration should be less than 1 mg/L (Basuyaux & Mathieu 1999). The higher concentration of TAN in the closed systems in the first month was caused by an increase in the backwash frequency of the bubble bead filters. An increase in frequency and intensity of the backwash harvested a higher amount of biofilm from the plastic beads or filter media, and thus decreased the ability of the biofilter to nitrify (Goltz et al. 1999, Sastry et al. 1999, Malone & Beecher 2000, Sandu et al. 2002). During the rest of the experiment, ammonia remained undetected or extremely low in all systems.

Nitrite has been shown to adversely affect growth or food consumption in several aquatic species; however, at least two different dose response patterns have been reported (Harris et al. 1997). A decrease in 50% in growth can be observed for the shrimp *Penaeus indicus* when exposed to 6.4 mg of NO_2/L, however, as nitrite concentration increased further, growth inhibition is not necessarily exacerbated, reaching a dose response plateau pattern (Wickins 1976, Chen & Chen 1992). In the abalone *H. tuberculata* (7–12 g), weight decreased up to 30% when 2 mg/L of NO_2 were present, and growth drastically decreased when nitrite concentration was higher than 5 mg/L (Basuyaux & Mathieu 1999). In our experiment, both closed systems S2 and S3, experienced a significantly higher nitrite concentration than S1 because they experienced a lower water exchange rate and the solids produced by abalone feeding activity on the macroalgae *Macrocystis pyrifera* were more difficult to remove. In January and March nitrite concentrations were detectable in both recirculating systems, whereas ammonia was not detected. This might be because of a decrease in the alkalinity values in these months, because nitrifying bacteria are especially sensitive to disturbances away from optimal alkaline conditions (Hagopian & Riley 1998). Probably if nitrates were measured, then more elements would be available to explain this discrepancy.

In early studies with the red abalone *H. rufescens* in a flow-through system, the reported alkalinity values were 95–140 mg/L $CaCO_3$ and 100–165 mg $CaCO_3$ in a closed recirculating system (Vivanco-Aranda 2004). In our experiment, the highest values for alkalinity in S1 were detected in the first two months (130 and 132 mg/L $CaCO_3$, respectively). For S2 and S3, although there was no significant difference throughout the experiment, the lowest values were detected in the first month.

One alternative to decrease the production costs associated with the pumping of high volumes of water is the use of recirculating systems. It is well known that a closed system must have less than 10% of water exchange per day of the total volume of the system (Losordo et al. 1992a, Malone et al. 1996, Masser et al. 1998). Several factors such as species cultured, density and management practices among others, will determine the optimum water exchange rate per day in a recirculating system (Timmons et al. 2002). In our experiment, the water exchange for S2 and S3 was less than 5% of the total volume and the only water replaced was that lost by evaporation and biofilter backflushes. One of the reasons we suspect abalone growth rates were significantly lower in S2 and S3, was because of the low water exchange and the presence of diverse metabolic wastes in the water (solids and NO_2) (Hahn 1989). An increase in the water exchange rate and better tank design would increase solid removal, improve growth rate and survival of *H. corrugata* in a closed recirculating system (Losordo et al. 1992b, Leonard et al. 2002, Pfeiffer 2004).

Growth rate in weight, shell length, and width in S1 was density dependent, D1 showed a higher growth rate than D2. For abalone cultured in flow-through systems, growth is inversely related to density (Mgaya & Mercer 1995, Capinpin et al. 1999, Valdés-Urriolagoitia 2000). Our results from S1 were similar to those obtained by other researchers that reported a reduction in growth ranging from 14–52% caused by 2- to 60-fold increase in density (Huchette et al. 2003). For *H. rufescens*, growth rate varies with density; 37.8 μm/day on 0.19 abalone/cm^2, 33.3 μm/day on 0.29 abalone/cm^2 and 19.44 μ/day on 0.41 abalone/cm^2 (Valdés-Urriolagoitia 2000). The abalone *H. discus hannai* grown in sea cages at high densities (3,000 abalone/m^2), showed a lower growth rate (70.2 μm/day and 11.11 mg/day) than abalone stocked at a lower density (1,000 abalone/m^2)(83.9 μm/day and 15 mg/day) and in each case, growth rate was independent from water temperature (Jee et al. 1988). Growth rate in weight, and shell length and width in both systems, S2 and S3 were lower than those obtained in S1, and showed not a clear trend in the effect of density on growth rates. In all systems and densities, (D1S1, D2S1, D2S1, D2S2, D1S3, D2S3) growth rates were higher than the 0.3 mm/month (0.0098 mm/d) reported for this species (Spencer 2002). Several factors can affect growth in juvenile abalone such as water flow and water quality variables like salinity, temperature, nitrogen

wastes, and tank design (Wickins 1981, Hindrum et al. 1995, Fleming at al. 1997, Harris et al.1998, Higham et al. 1998). Even when food was not a limiting factor in any of the three holding systems, the solids generated by leftover macroalgae were difficult to remove and tended to accumulate in the tanks S2 and S3. In coldwater fish such as rainbow trout, the presence of suspended solids in water results in poor growth and stress (Timmons et al. 2002). Nitrite and temperature were the only two water quality parameters that greatly differed between the flow-through and recirculating systems. Nitrite chronic exposure probably affected growth rate of *H. corrugata* in both recirculating systems, S2 and S3. As the concentration of nitrite increase, an apparent decline in oxygen consumption can compromise respiratory efficiency (Harris et al. 1997). In another study on the carp *Cyprinus carpio*, a chronic exposure to nitrite increases the levels of methemoglobin, decreasing the levels of arterial oxygen (Jensen et al. 1987, Williams et al. 1992). Harris et al. (1997) suggested that nitrite can depress growth by increasing protein catabolism, as indicated by a higher ammonia excretion. The greenlip *H. laevigata* is more sensitive to nitrite than other aquatic species, suggesting that other abalones species such as *H. corrugata* could show the same metabolic response (Harris et al. 1997).

The highest mortality rates were observed at the beginning of the experiment, probably caused by stress from manipulation, water temperature variation, and higher nitrite concentration (Edwards et al. 2000, Ragg et al. 2000, Spencer 2002). Similar results were observed for the abalone *H. discus hannai* cultured in a recirculation system, where the highest mortality rate was observed in the first 70 days (Nie et al. 1996). On several days we experienced temperatures equal or higher than 20°C, which caused mortality on the abalones. Re-Araujo (2003) reported a 100% survival of *H. corrugata* at 24.5°C, but in our experiment abalone showed a better growth rate and higher survival rate when temperature was around 17°C. The survival rate reported for this species is of 74% in a flow-through system (Spencer 2002). In our experiment, the lowest survival rate (71.7%) was in the recirculating system S3 with an average water exchange rate of less than 3%. In another experiment using two different biofiltration systems with the abalone *H. discus hannai* a survival was 66%, and 63.4% was attained (Nie et al. 1996). The effects of manipulation at the beginning of the experiment and water quality management were the factors that have a profound effect of growth and survival of *H. corrugata*. In the abalone *H. laevigata*, low ammonia affected shell growth more than weight, whereas in high ammonia concentrations, an inverse effect was observed (Harris et al. 1997). In our experiment body weight was more affected than shell growth. Similar effects in length gain/weight gain because of nitrite exposure were reported by Liu & Avault (1996).

The data obtained in this experiment showed that it is possible to grow pink abalone in recirculating systems. However the tank design and water inlets and outlets did not allow an efficient solid removal and the water exchange rates were probably too low. These factors probably produced lower growth rates than in the flow through system. More research is required to address these issues.

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