

Water Quality Management with *Bacillus* spp. in the High-Density Culture of Red-Parrot Fish *Cichlasoma citrinellum* × *C. synspilum*

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Abstract.—Red-parrot fish (male midas cichlid *Cichlasoma citrinellum* × female redhead cichlid *C. synspilum*) is one of the most important tropical fish in Taiwan. In high-density culture, poor water quality often induces emaciation, gill filament ulceration, gill opercula malformation, and high mortality. In an effort to solve these problems in a high-density culture environment, two bacteria, *Bacillus subtilis* and *B. megaterium*, were added to the recirculating-water systems of the grow-out facilities. The addition of *Bacillus* held total ammonia nitrogen concentrations to 5.4 mg/L or less and chemical oxygen demands to 40.8 mg/L or less; it also maintained the transparency of the recirculated aquarium water at 30–50 cm. Survival rate and quality of fish were dramatically improved with this microbial water quality management process. This method is easy to perform and can be adapted effectively in the commercial culture of red-parrot fish.

Tropical fish culture is an important aquatic industry in Taiwan. Production of the red-parrot fish (Figure 1) was worth more than US\$3.2 million dollars (about 0.8 billion Taiwanese dollars) in 1990, or almost 20% of the tropical fish production in Taiwan (Chung 1992). Because of water temperature limitations, the major grow-out facilities are located in southern Taiwan, where red-parrot fish are reared in the outdoor ponds without recirculating-water systems. However, hatcheries are located in northern Taiwan, where red-parrot fish are hatched and grown in greenhouses. It is well documented that tropical fish reared at high densities in greenhouses require effective recirculating-water systems (Chung 1992).

The red-parrot fish were initially produced in a cross between male midas cichlids *Cichlasoma citrinellum* and female redhead cichlids *C. synspilum* (also known in Taiwan as purple-red fire mouth fish) in Taipei, Taiwan, in 1989 (Konings 1989; Axelrod et al. 1990; Chen 1990a, 1990b, 1990c). The body colors of the fish vary from pinkish red

to blood red, the body shape is somewhat truncated with a thick foreback (the back before dorsal fin), and the mouth is triangular and does not close.

In greenhouses, red-parrot fish are cultured at high density in 250–300-L aquaria. Approximately 1,000 young fish at a density of 8.23 g/L or 400 adult fish (average body length = 12.7 cm) at a density of 135 g/L are cultured in a single aquarium. Because of the heavy daily feeding schedule (7.5% body weight for juveniles and 3.2–5.5% for adults), in grow-out facilities, total ammonia nitrogen concentration is always above 7.3 mg/L, and the chemical oxygen demand is above 80 mg/L (Chen 1998). Therefore, water is exchanged at 4-d intervals. In cold weather, however, water temperatures may decrease by 5–7°C during the water exchange, which can stress fish and may initiate diseases and disturb the reproductive cycle of spawning pairs.

Because water quality in the high-density hatcheries or grow-out facilities is poor, fish frequently suffer gill diseases caused by *Cytophaga columnaris* and *Aeromonas hydrophila*. Malformation of gill opercula caused by swelling of gill filaments is often observed and is caused by the infections of *A. hydrophila* and some gram-negative bacteria (Jung 1993). Mortalities can be about 50% for the young and 35% for adults (Chen 1998). Therefore, management of good water quality is important. Species of *Bacillus* have been extensively used in the water quality management of aquaculture and wastewater treatment, often to remove organic substances and ammonia in aquarium water and wastewater (Boyd 1982; Pritchard and Bourquin 1984; Chen et al. 1991; Chen 1992). We tested the use of two *Bacillus* species, *B. subtilis* and *B. megaterium*, in the water quality management of high-density culture of red-parrot fish.

Methods

Recirculation system.—We used four identical recirculating-water systems for the experiments (Figure 2), two replicates for the treated group and two for the control group.

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FIGURE 1.—The red-parrot fish (male midas cichlid *Cichlasoma citrinellum* × female redhead cichlid *C. synspilum*); its body colors are pinkish red to blood red, the body shape is robust, the foreback is thick, and the mouth is triangular and does not close.

Two additional systems were used for acclimation. Each recirculating-water system included 19 glass aquaria (120 × 60 × 45 cm, filled to a depth of 40 cm); 14 aquaria were used for rearing fish and 5 for filtering. The filtering aquaria were filled with nylon net (mesh size = 2 mm) as filters and connected by large soft hoses (diameter = 7.0 cm). All the systems shared the same water supply. In the recirculating-water systems, water was transferred (0.25-hp [186.5-W] water pump; flow rate of 1.6 L/min) from one filtering aquarium (the final one in the series of filtering aquaria) into seven rearing aquaria located above the others. Water then flowed into seven rearing aquaria below them at the middle level and subsequently into the five filtering aquaria at the bottom level. The rearing aquaria and filters were cleaned once per week.

Sampling and culture conditions.—Young red-parrot fish, 2.19 ± 0.73 g (mean \pm SD) in weight and 3.23 ± 0.64 cm in total length, were collected from the same grow-out facility. After a 10-d acclimation period in two recirculating-water systems, fish were randomly distributed into four new recirculating-water systems. Each rearing aquarium received 300 fish; and each recirculating-water system received 4,200 fish. For all four recirculating-water systems we used 16,800 young red-parrot fish or a density of 2.28 g/L.

The fish were initially fed commercial sinking

pellets (32.0% crude protein, 12.5% carbohydrate, 6.7% crude fiber, 6.0% crude fat, 10.5% ash, and 6.0% moisture) at 4.5% body weight twice a day. Size of the granule was increased from 0.85 to 1.5 mm as the fish grew. When fish reached about 5.0 cm in total length, floating pellets (2.5–3.8 mm; 35.0% crude protein, 12.5% carbohydrate, 6.8% crude fiber, 8.0% crude fat, 14.5% ash, and 6.0% moisture) were used; feeding rate was reduced to 2.0% body weight twice a day. Mean body weight of the fish was determined by sampling 10 fish from each of three aquaria selected without known bias from each recirculating-water system.

Bacterial culture.—Chen (1992) reported that adding *B. subtilis* and *B. megaterium* twice a week can remove significant amounts of ammonia nitrogen from an aquarium. We prepared separate bacterial cultures of *B. subtilis* and *B. megaterium* in TSB (tryptic soy broth; Difco) at room temperature for 24 h with continuously shaking (Sneath et al. 1986; Parry et al. 1988). Bacterial density greater than 10^7 colony-forming units (cfu)/mL in each culture was confirmed by the serial dilution method (Austin 1988), and 2.5 L of each culture was mixed and sprayed twice a week directly into the filtering aquaria of the appropriate recirculating-water systems. The experiments were performed with two replicates. No bacteria were added to the control group.

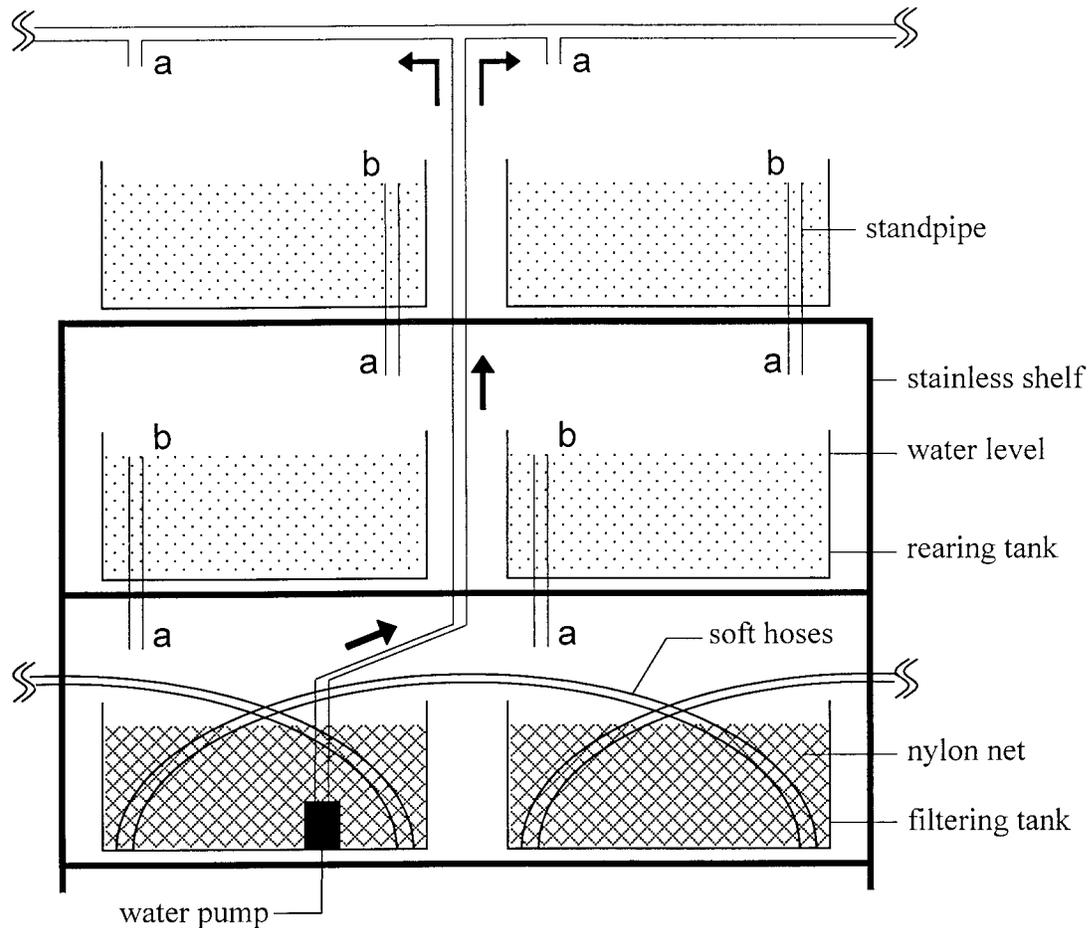


FIGURE 2.—Diagram of the recirculating-water systems (iterative aquaria excluded); arrows indicate the direction of flow, (a) indicates inflow locations, and (b) indicates outflow locations. In each of the four systems, the 14 rearing aquaria were situated at the upper and middle levels, and the 5 filtering aquaria were situated at the bottom level. Recirculated water was pumped from the bottom level to the top level and returned by gravity flow to the bottom level. The filtering aquaria were connected by soft hoses and were filled with nylon netting that served as the filters. All the aquaria were aerated.

Total bacterial numbers and numbers of *Bacillus* spp. in water samples from the bacteria-treated and the control groups were determined by the serial dilution assay method. All the single colonies on the culture plates were enriched individually and subsequently subjected to six biochemical tests to identify to the genus *Bacillus* (Sneath et al. 1986; Parry et al. 1988).

Biochemical and biological assays.—Water temperature was maintained at 28.0°C (Axelrod 1989; Heinen et al. 1996) by air conditioning and an electric heater in the water. Water temperature, dissolved oxygen, pH, concentration of total ammonia nitrogen (TAN), chemical oxygen demand (COD), and transparency of the water samples

were determined once a week before cleaning the recirculating-water systems.

The TAN was determined with a test-kit manufactured by Palintest, Ltd., England, and the COD with a test-kit by Kyoritsu, Chemical-Check Laboratory Corporation, Japan (Linore et al. 1989). The transparency meter (Sin-An instrument Co., Taipei, Taiwan) was a glass cylinder with a cross mark at the bottom and an outlet near the bottom. Water samples for testing were loaded into the cylinder and slowly drained from the outlet until the cross mark became visible. Depth of the remaining water within the cylinder was defined as the transparency.

Health of the fish was closely monitored, es-

TABLE 1.—Numbers of the dead red-parrot fish in the four recirculating systems. In the eighth and eleventh weeks, fish in the control group became diseased and suffered heavy mortality. At the beginning of the experiment, each recirculating-water system received 4,200 fish. Values in parentheses are mortality rates (%).

Weeks	Numbers of dead fish			
	Bacteria-treated		Control	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	15	12	76	47
2	7	19	67	59
3	10	6	32	47
4	6	3	49	32
5	6	3	73	67
6	8	7	93	59
7	12	10	44	67
8	18	16	141	89
9	10	7	46	59
10	12	14	117	101
11	10	15	127	110
12	6	7	114	127
13	7	11	125	98
14	10	12	113	72
15	17	11	87	69
Total	154 (3.67)	153 (3.64)	1,304 (31.05)	1,103 (26.26)

pecially on the skin and gills. Diseases affecting these areas may cause color change, body shape malformation, or abnormal behavior. When poor health was observed, exchanging water and cleaning filters was the first treatment. If the situation did not improve, a treatment with a mixture of 6% methylene blue, 2% malachite green, and 20% chloramphenicol (Wonder Pharmaceutical Co., Tainan, Taiwan) was applied at the dosage of 15 mg/L (Nelson et al. 1979; Wang 1987).

Statistical analysis.—A two-tailed unpaired *t*-test was performed on the tank performance means (i.e., body weight, TAN, COD, and transparency) to determine whether differences within the replicates and between the bacteria-treated and control groups were statistically significant ($\alpha = 0.05$).

Results

Throughout the 105-d experimental period, water temperatures were maintained at 24.2–28.5°C. The pH values were 6.25–8.0 mg/L, and dissolved oxygen was 4.7–6.8 mg/L. The final mortality rate was 3.65% for the bacteria-treated group and 28.65% for the control group (Table 1).

Red-parrot fish in the bacteria-treated group gained more body weight than those in the control group (Figure 3). At the end of the experiment,

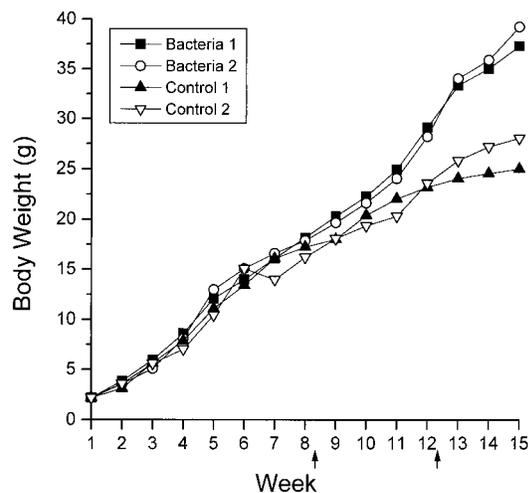


FIGURE 3.—Body weights of the fish sampled from the four recirculating-water systems. The bacteria-treated groups received *Bacillus subtilis* and *B. megaterium*; control groups did not. By the end of the experiment fish in the bacteria-treated groups gained 45% more body weight than those in the control groups ($P < 0.005$). Arrows indicate times at which drugs were administered to the control groups.

mean body weight (\pm SD) of two replicates in the bacteria-treated group was 38.3 ± 6.4 g and mean total length was 7.4 ± 1.2 cm. In contrast, mean body weight of the fish in the control group was 26.8 ± 8.3 g and mean total length was 6.7 ± 1.0 cm. Body weight differences of the replicates were not significant between the two bacteria-treated groups ($t = 0.015$, $P > 0.1$) nor between the two control groups ($t = 0.057$, $P > 0.1$). The overall body weight differences between the bacteria-treated and the control groups were not significant ($t = 1.268$, $P > 0.1$). However, the body weight differences between the two groups in the last 5 weeks of the experiment were significant ($t = 4.275$, $P < 0.005$). At the end of the experiment, fish in the bacteria-treated groups gained 45% more weight than those in the control groups ($t = 5.981$, $P < 0.005$), which were more elongate and less robust, lacking the fatty shape in the foreback and abdomen.

In the bacteria-treated groups, TAN was 0.7–5.4 mg/L; in the control group TAN was 0.9–11.3 mg/L (Figure 4). Differences in TAN were not significant between the bacteria-treated replicates ($t=0.258$, $P > 0.1$) nor between the control replicates ($t=0.983$, $P > 0.1$). The TAN differences between the bacteria-treated and the control groups were significant ($t = 6.764$, $P < 0.005$). In the

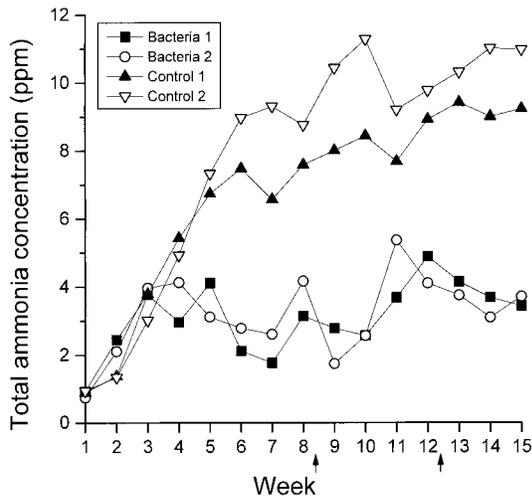


FIGURE 4.—Total ammonia nitrogen (TAN) concentration of the water sampled from the four recirculating-water systems used for rearing red-parrot fish. In the control group, the TAN increased throughout the experiment. In contrast, the TAN in the bacteria-treated group remained at a lower level throughout the experiment. Arrows indicate times at which drugs were administered to the control groups.

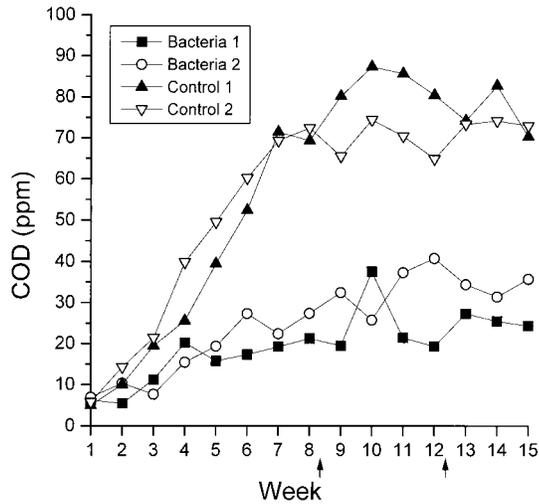


FIGURE 5.—Chemical oxygen demand (COD) of the water sampled from the four recirculating-water systems used for rearing red-parrot fish. In the control group, the COD increased throughout the experiment. In contrast, the COD in the bacteria-treated group remained at a lower level throughout the experiment. Arrows indicate times at which drugs were administered to the control groups.

bacteria-treated group, TAN in the two recirculating-water systems increased sharply during the first 3 weeks; afterwards, TAN remained at about the same low level. However, TAN in the control group increased sharply during the first 6 weeks and continued to increase until the end of the experiment.

The COD values in the bacteria-treated groups were 5.4–40.8 mg/L; in the control groups, COD was 5.0–87.4 mg/L (Figure 5). The variation of COD was similar to that of TAN. The COD differences between the bacteria-treated replicates were not significant ($t=1.581$, $P > 0.1$), nor were they in the control groups ($t=0.172$, $P > 0.1$). The COD differences between the bacteria-treated and control groups were significant ($t = 6.631$, $P < 0.005$). The COD of the control group increased sharply during the first 7 weeks and continued to increase until the end of the experiment. In contrast, the COD in the bacteria-treated group was maintained at a lower level throughout the experiment.

Transparencies of the bacteria-treated groups were 30.4–49.8 cm; in the control groups values were 14.3–53.9 cm (Figure 6). Transparency differences between the bacteria-treated replicates were not significant ($t=0.720$, $P > 0.1$), nor were they in the control groups ($t=0.518$, $P > 0.1$). The

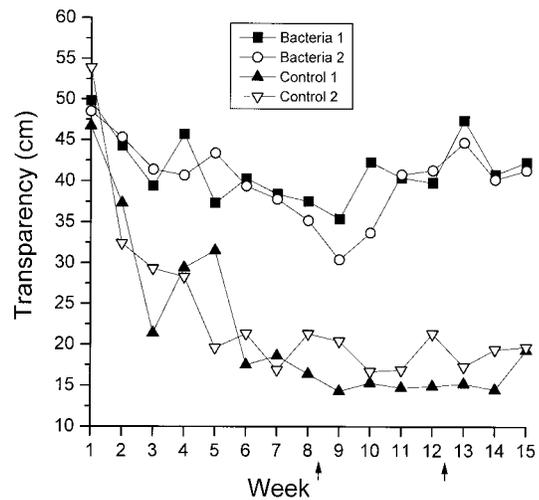


FIGURE 6.—Transparency of the water sampled from the four recirculating-water systems used for rearing red-parrot fish. In the bacteria-treated group, the transparency remained high throughout the experiment. In contrast, the transparency in the control groups decreased throughout the experiment. Arrows indicate times at which drugs were administered to the control groups.

TABLE 2.—Total bacteria count and number of *Bacillus* spp. in water samples. In the bacteria-treated group, the *Bacillus* spp. predominated after 3 weeks. In the control group, bacteria numbers increased sharply at weeks 8 and 11 (mainly gram-negative rods) and there were serious disease outbreaks in this group.

Weeks	Bacteria-treated				Control	
	Replicate 1		Replicate 2		Replicate 1	Replicate 2
	<i>Bacillus</i> spp. (%)	Total	<i>Bacillus</i> spp. (%)	Total	Total	Total
1	1.0×10^2 (10.3)	9.7×10^2	1.1×10^2 (5.2)	2.1×10^3	1.1×10^2	1.2×10^2
2	2.3×10^2 (10.4)	2.3×10^3	3.1×10^2 (12.9)	2.4×10^3	1.2×10^3	1.7×10^3
3	4.3×10^2 (17.9)	2.4×10^3	3.3×10^2 (12.2)	2.7×10^3	4.9×10^3	6.9×10^3
4	1.2×10^3 (30.8)	3.9×10^3	1.3×10^3 (40.6)	3.2×10^3	3.1×10^2	3.4×10^3
5	1.9×10^3 (38.3)	4.9×10^3	7.9×10^3 (60.8)	1.3×10^4	4.7×10^3	5.4×10^3
6	6.9×10^3 (49.3)	1.4×10^4	3.1×10^4 (48.4)	6.4×10^4	6.7×10^3	4.1×10^3
7	3.1×10^4 (33.0)	9.4×10^4	9.2×10^4 (70.8)	1.3×10^5	1.2×10^4	6.7×10^3
8	8.4×10^4 (70.0)	1.2×10^5	9.1×10^4 (47.9)	1.9×10^5	1.4×10^4	9.2×10^4
9	3.1×10^5 (63.3)	4.9×10^5	3.1×10^5 (32.3)	9.6×10^5	6.4×10^4	5.3×10^4
10	9.7×10^4 (51.1)	1.9×10^5	3.9×10^5 (41.5)	9.4×10^5	7.3×10^4	8.9×10^4
11	1.2×10^5 (57.1)	2.1×10^5	3.2×10^5 (76.2)	4.2×10^5	1.4×10^5	1.1×10^5
12	3.1×10^5 (57.4)	5.4×10^5	3.1×10^5 (48.4)	6.4×10^5	6.3×10^4	9.4×10^4
13	2.9×10^5 (61.7)	4.7×10^5	2.7×10^5 (45.8)	5.9×10^5	4.2×10^3	4.9×10^4
14	2.3×10^5 (56.1)	4.1×10^5	3.4×10^5 (65.4)	5.2×10^5	3.7×10^4	5.9×10^4
15	2.6×10^5 (41.3)	6.3×10^5	3.9×10^5 (60.9)	6.4×10^5	1.2×10^5	7.4×10^4

transparency differences between the bacteria-treated and the control groups were significant ($t=9.406$, $P < 0.005$). Transparencies of the bacteria-treated and the control groups started to show a difference in the second week and showed a more obvious difference in the sixth week. The transparency of the bacteria-treated groups remained at about 40 cm compared with 15 cm in the control groups. Water exchange and filter cleaning can increase the transparency immediately in this experiment.

The total bacteria count in the water samples of all recirculating-water systems increased from 10^2 to 10^6 cfu/mL (Table 2). In the first 3 weeks, the ratio of *Bacillus* spp. in the bacteria-treated group was lower than 20%. Subsequently, *Bacillus* spp. became predominant in the water, the ratio increasing to 30–70%. In contrast, the total bacteria count in the control group increased to more than 10^5 cfu/mL in the eleventh week, Gram-negative rods being the predominant. Fish in this group suffered serious diseases and mass mortality (Table 1).

There were no disease symptoms in the bacteria-treated group. Fish in this group were healthy and fed actively. Fish in the control group, however, by the sixth week showed erratic behaviors, often came to the water surface, and fed much slower and less actively. When this occurred (week 6), exchanging water and cleaning filters improved their health. However, the fish in control group developed diseases twice more during the experiment. Exchanging the recirculating water, clean-

ing the filters, and stopping feeding did not improve the health of fish.

Several strains of pathogenic bacteria were isolated from the gill filaments and body mucus of the infected fish in the control group. The predominant species among these bacteria were *C. columnaris* and *A. hydrophila* (Krieg and Holt 1984; Rahman et al. 1997), which are often found in tropical fish and water environments (MacFaddin 1980; Post 1987; Wang 1987; Jung 1993). The remaining species were almost all gram-negative rods. The major symptoms were ulceration and swelling in the gill filaments.

Discussion

Throughout the experiment, the concentration of dissolved oxygen (4.7–6.8 mg/L), water temperature (24.2–28.5°C), and pH (6.25–8.0) were suitable for culture of cichlid fish (Boyd 1982). Upon completion, fish in the bacteria-treated group were 45% larger than those in the control group (Figure 3), and their body surface was smooth, bright, and shiny.

High TAN reduces the growth rate and causes disease in shrimp and commercial fish culture (Chen et al. 1991). In the control group, after 5 weeks, TAN was up to 7 mg/L, increasing to 10 mg/L after 9 weeks (Figure 4). Fish can survive at TAN of 10 mg/L (Alabaster and Lloyd 1982; Abel 1989; Chiayvareesajja and Boyd 1993), as did the red-parrot fish in the control group that experienced this concentration. However, feeding was less active in these controls, disease was more

common, and mortality was higher. In the early stage of culture, exchanging water and cleaning filters improved the conditions after stress first appeared. However, the fish in the control group got sick in the eighth and twelfth weeks, and exchanging water and cleaning filters did not improve upon their health. Eventually, we had to stop feeding for 2 d and administer medication to improve their health and thwart additional loss. Nevertheless, about 30% of the control fish eventually succumbed. It implies that concentrations of TAN can affect the health of fish in a recirculating-water system.

Water transparency was determined by the mass of suspended matters in water. Because the suspended matter was mainly constituted by organic particles from food pellets and fish waste, the variation of COD concentration may be related to the mass of suspension.

In the bacteria-treated group, almost all particles were trapped in filters and easily removed by periodic cleaning; water transparency remained high in this group. In contrast, suspensions in the control group could not be effectively trapped by the filters, transparency remained low, and water was colored white to yellow. This led us to conclude that *Bacillus* spp. can reduce the COD and increase the transparency effectively in the aquatic environment.

In the bacteria-treated group, the added bacteria became the predominant species isolated from the water. Even though the *Bacillus* spp. solution was added twice a week, the concentration of the *Bacillus* remained between 10^4 and 10^5 cfu/mL, which may be the approximate maximum concentration of *Bacillus* in this kind of recirculating-water system. That is, the concentration of *Bacillus* is limited by the niche types available in aquatic environments (Rheinheimer 1985). The lower levels of TAN and COD and the higher transparency in the bacteria-treated group were attributed the presence of *Bacillus* spp., which were the major species in the water environment of the bacteria-treated group (as described by Chen 1992).

Red-parrot fish in the control group had serious health problems, including scale loss, gill filament ulceration, gill opercula malformation, and poor condition. Medications were applied to control the diseases effected by *C. columnaris*, *A. hydrophila*, and several strains of gram-negative rods that were isolated from the sick fish. Nevertheless, mortality in the control group was as high as 30%. In the later periods of the experiment, the cultural density in the control group was lower than that in the

bacteria-treated group, but the water quality of the control group was still much worse than that of the treated group, which remained healthy. Because *Bacillus* spp. was the predominant species of the bacteria phase in the bacteria-treated group, we concluded that the *Bacillus* spp. not only improves the water quality but also inhibits pathogenic bacterial outbreaks.

In summary, treatment of aquaculture recirculating water with *Bacillus* spp. can effectively decrease the TAN and COD and improve environmental conditions for red-parrot fish. This type of water quality management is cost-effective and easy to perform. Fish quality and overall harvest can be dramatically improved by managing water quality with *Bacillus* spp.

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