

# **Changes in Water Quality and Performance of a Closed Recirculating Aquaculture System for Pejerrey (Atlantic Silversides)**

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## **Introduction**

Terminology for recirculating systems is ambiguous. As used in this paper, “Closed” means no continuous drainage, and water addition was restricted to the replacement of evaporation and water removed along with the sludge only.

Closed recirculating aquaculture is desirable due to limited environmental impact and expanded number of possible production sites. For example, economic water temperature and water quality management are possible, and the culture environment fitted to many different species can be created easily in any locale.

Japan has some of the highest energy costs in the developed world, hence energy cost reduction is particularly important here. However, if water can be recirculated, production sites far from oceans and rivers, on cheap land which wasn't available for aquaculture production thus far can be made into fine production areas. Waste heat from refuse incineration and geothermal energy are widely available in Japan. If this can be harvested, it is believed that economic land-based production of many species will become possible in Japan.

Nitrate, as the final product of the nitrification process, accumulates continuously in closed systems and can reach quite high concentrations if unchecked. The nitrate toxicity level for pejerrey is still unclear. However, high levels are reported to impact growth rates with other species (Muir and Roberts, 1982), and high concentration inculture water of low alkalinity and low pH were shown to adversely affect octopus respiration (Hirayama, 1966). Therefore, denitrification is necessary for long term stability in a closed recirculating aquaculture systems. However, even without a specialized denitrification unit, if local anaerobic conditions occur in the system, denitrification bacteria may grow and some denitrification may occur. This often results in undesirable by-products such as hydrogen sulfide production so this isn't desirable in commercial systems.

In this experiment we attempted to decrease the nitrate concentration via a denitrification unit while keeping the rearing environment optimal for the fish with advanced solids removal technology and an aerobic nitrification filter with stable operation for 1 year. This paper reports on the water treatment processes for this system, and on the relation of the accumulation of the nitrate and water exchange rate.

## Materials and Methods

### System and its operation

The system consisted of two particle-trap equipped octagonal tanks ( $0.75 \text{ m}^3$ ) for the rapid collection of feces and uneaten feed, a mechanical filter, two UV units (40 W), a biological filter which provided nitrification, two hollow-fiber oxygen injection systems, two pumps (0.2 kw) and an alarm device (Fig.1). A foam fractionation unit and drum filter were used for mechanical filtration. A rotating biological contactor (RBC) and

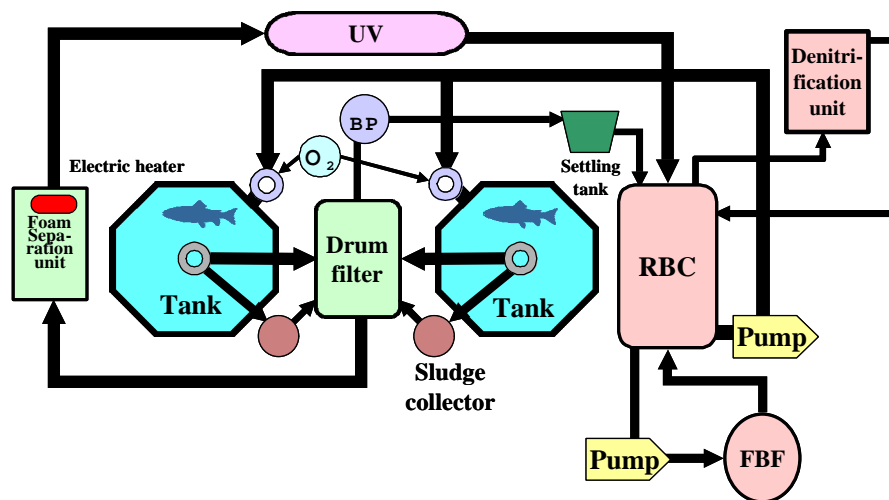


Fig. 1. Schematic view of closed recirculating aquaculture system.

fluidized bed filter were used for the biological filter. The denitrification unit ( $0.12 \text{ m}^3$ ) was connected to the system on day 180. Water temperature regulation was done via electric heater (2 kw). Total system volume was about  $2.1 \text{ m}^3$ . The flow rate was fixed at

2.1 m<sup>3</sup>/h.

At the bottom of each tank a particle trap (ECO-TRAP, AquaOptima A/S, Norway.) was used to remove solid waste promptly from the rearing environment. The effluent is then divided into two streams, a low volume/ high solids stream and a high volume/ low solids stream. The latter flows to the drum filter from the tank center. Uneaten feed that settled in the tank, and excrement are carried to the sludge collector, where it sediments, and the clarified flow from the sludge collector flows to the drum filter. Uneaten feed and excrement that were deposited in the sludge collector were discharged from the basin daily. The drum filter (0.1 m<sup>3</sup>, Harry Fischer, Inc., USA) was a self-cleaning screen filter with a 45 µm mesh screen. The captured solids were sucked from the filter with a vacuum pump and carried to settling tank (0.02 m<sup>3</sup>, 0.03 m<sup>3</sup>/h). The clarified flow from the settling tank was led to the RBC. Solids beyond 45 µm were eliminated by the drum filter, and the screened water flowed by gravity in order into the foam separation unit, the UV unit and the biological filter.

The foam fractionation unit operated from the culture initiation until the 305th day. But, it we discontinued operation after day 306 because stable foam didn't arise and the elimination of waste was not observed.

The RBC media was interlocked fiber (SaranLock OM-150, Asahi Chemical Industry, Inc.). The specific area of the media was 410 m<sup>2</sup>/m<sup>3</sup>. The fluidized bed filter media was sand (0.01 m<sup>3</sup>). The specific area of the media was 8000 m<sup>2</sup>/m<sup>3</sup>. Fritz-Zyme NO.9 (Fritz Chemical, Inc.) that contained the marine nitrification bacteria was added both in the RBC and the fluidized bed filter on day 45, and operated prior to the culture initiation.

The denitrification unit was designed as a submerged filter (0.16 m<sup>3</sup>/day). The media was fiber (SaranLock CS-100, Asahi Chemical Industry, Inc.). The specific area of the media was 740 m<sup>2</sup>/m<sup>3</sup>. A denitrification unit was connected to the RBC after the preliminary operation for 6 days on day 180. Glucose was added to the denitrification unit inflow water as a 5 % (w/v) aqueous solution from day 190 until day 265, and the glucose concentration of the inflow water was controlled as an organic carbon source in the range of 0-270 mg/l. From day 280 to 365, 10 % (w/v) methanol solution were added, and the methanol concentration of the inflow water was controlled in the range of 0-666 mg/l. Inoculation of denitrification bacteria used the marine denitrifying bacteria *Alcaligenes* sp. Ab-A-1 strain (Watanabe et al., 1991).

Dechlorinated tap water was used to make culture water by mixing with artificial seawater formulation (New MarineArt, Tomita Pharmaceuticals Co., Ltd.) to 7 ‰. Tap water was used to replenish evaporation losses and artificial seawater was used to replenish losses of water with sludge disposal. No continuous water replacement was undertaken. The water was pumped and processed through two hollow-fiber oxygen injection columns before re-entering the tank, and dissolved oxygen levels of each tank were individually controlled to 100 % saturation. 5 % (w/v) NaHCO<sub>3</sub> solution and 5 % (v/v) hydrochloric acid solution were added as needed for pH adjustment to maintain pH 7-8.2. Culture water temperature in the tank was set to 24 °C.

## Culture conditions

On August 1, 1997, 383 Pejerrey (*Odonthestes bonariensis*, average weight 1.57 g) were stocked in the tank, and were reared for 1 year. Commercial feed for flounder was used for the majority of the experiment, and pellet size was changed in accordance with the growth. Feeding was controlled at intervals of 10-20 minutes every day from AM 4:00 until PM 22:00 and feeding amount was measured by a load cell everyday. Feeding to just above satiation level was maintained. Culture conditions for the year are shown in table 2. At the end of experiment, the average weight was 109.1 g, and the survival rate was high at 92.2 % and the final culture density (the rate of the fish total weight toward the water weight of the tank) was 2.7 %.

## Sample analysis

Measurements of water temperature were taken every two hours by temp. logger (Type 0610.1720, Test term Inc.). pH readings were taken with a pH meter (GP-1D, Pasolina Inc.) daily. Dissolved oxygen was measured continuously with a DO meter (OXY2100, Danfoss Inc., TOX-90, Toko Chemical Laboratories Inc.). The water samples were analyzed for NH<sub>4</sub>-N (Indophenol method), NO<sub>2</sub>-N (Diazotization method), NO<sub>3</sub>-N (Cadmium Reduction method), salinity (Silver Nitrate Standard Solution) and alkalinity, and a spectrophotometer SP-20 (Shimadzu Inc.) was used for analysis. The Water for analyses was sampled from the tank effluent at 9 o'clock every morning, and analyzed in our daytime.

To characterize the performance of the system's treatment units, a series of efficiency tests were conducted. The units included the tank's particle traps, the RBC, the fluidized bed filter, and the denitrification unit. For these efficiency tests, inflow and outflow samples were taken at each unit, and were analyzed for NH<sub>4</sub>-N. The NH<sub>4</sub>-N removal rates were calculated from the flow rates. Sediments in the sludge collector and the drum filter settling tank were filtered with the screen mesh of 1.5 mm size to eliminate uneaten feed, and were analyzed for Total Kjeldahl Nitrogen (TKN). After uneaten feed was removed with a mesh screen, dry weight was determined. Food consumption weight of the fish was calculated by deducting uneaten feed weight from the feeding weight, allowing for pellet moisture content and the nitrogen consumption was calculated from the nitrogen content of the feed.

## Results

Average body weight was 109.2 g, average total length was 22.9 cm and the density of the total weight per tank water volume reached 26.5 kg/m<sup>3</sup> at the end of experiment. Survival rate remained very high, exceeding 92 % at the end of one year.

The water temperature was kept stable at 23.8± 0.7 °C (n=4372, 21.2-28.2 °C), near the objective water temperature (24.0 °C). The pH was mean 7.60±0.26 (n=265, 7.00-8.18), the alkalinity was mean 2.15±1.6 meq/l (n=274, 0.65-7.27). The cumulative added amount of NaHCO<sub>3</sub> was 14.1 kg, the cumulative added volume of the hydrochloric acid

became 2.47 l. Dissolved oxygen (DO) averaged  $103.5 \pm 3.0$  % ( $n=365$ , 95.7-135.1 %) of the saturation values, and well controlled to the target level. (100 %). Salinity concentration became mean  $7.4 \pm 0.3$  ‰ ( $n=162$ , 6.8-8.6 ‰). The mean total ammonia nitrogen  $\text{NH}_4\text{-N}$  concentration was  $0.18 \pm 0.07$  mg/l ( $n=358$ , ND (no detection)-0.36 mg/l,  $\text{ND} < 0.05$  mg/l), and was stable through the year, and it could be maintained low. The mean unionized  $\text{NH}_3\text{-N}$  concentration was  $0.004 \pm 0.003$  mg/l ( $n=264$ , ND-0.016 mg/l,  $\text{ND} < 0.001$  mg/l) during the culture that it was calculated from the pH, water temperature and TAN (Bower and Bidwell, 1978). The mean  $\text{NO}_2\text{-N}$  concentration was  $0.18 \pm 0.12$  mg/l ( $n=358$ , ND-0.77 mg/l,  $\text{ND} < 0.01$  mg/l). (Fig.2)

Accumulation of nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) in the culture water continued (Fig.2) until the 190th day from the start of the experiment. A denitrification unit was connected to the system on day 180. From about the 280th day, when methanol solution was added, denitrification began to occur remarkably, and  $\text{NO}_3\text{-N}$  concentration reached a peak of 900 mg/l until then. Denitrification proceeded rapidly from the 300th day, and declined to

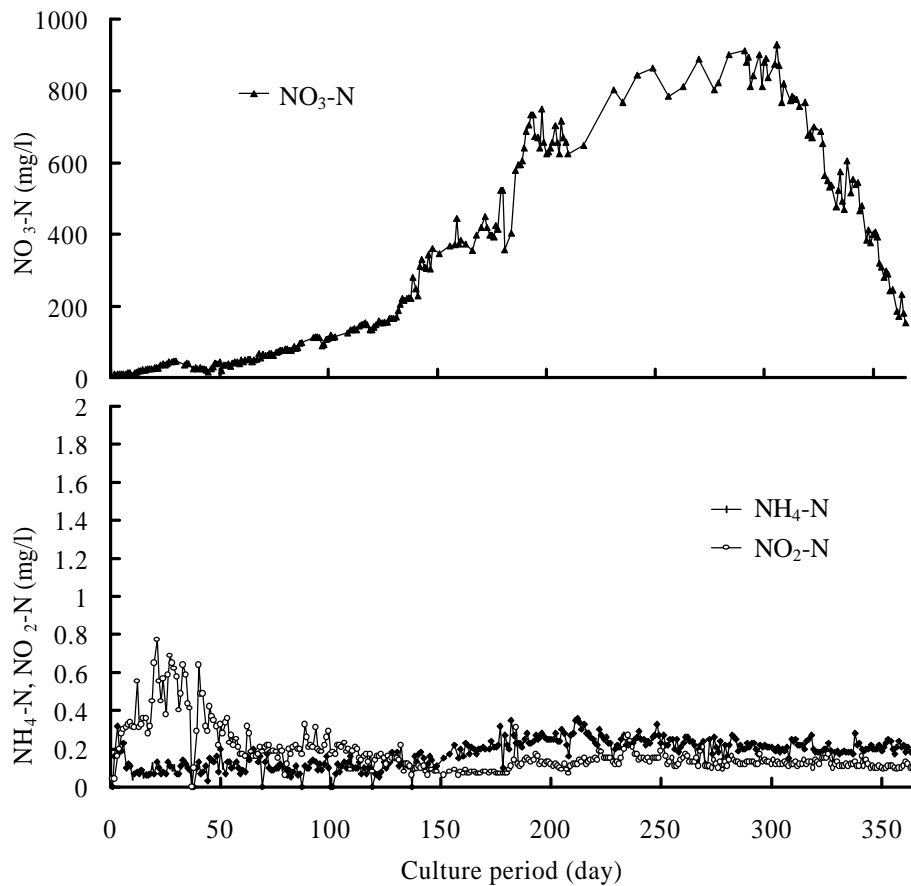


Fig. 2. Changes in concentrations of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  in the culture water.

150 mg/l by the time of the experiment completion. The denitrification rate peaked at 48 g-N/day and the specific denitrification rate was 400 g-N/m<sup>3</sup> filter media/day (specific

surface area 740 m<sup>2</sup>/m<sup>3</sup>). The concentration relationship was 0.47 mg-N/mg-CH<sub>3</sub>OH and alkalinity production was 0.045 meq/mg-NO<sub>3</sub>-N (Yoshino et al., 1999).

Intensive replacement of water was not undertaken with this system. However, an average of 10 l (0.5 % of the total water volume) were added per day as make-up for water lost with the dumping of the sludge from the sludge collectors and evaporation.

The nutrient process rate of each unit

The average food consumption weight per hour, and nitrogen process volume as ammonia and excrement with each unit are shown in Table 1. As for total food consumption weight per day mean 188.5±11.7 g (n=4175, 5-203.3 g), provisioning time was 18 hours/day. The food consumption rate was 10.5g/h, and the nitrogen ingestion rate of 744±46 mg-N/h was calculated. Of ingested nitrogen, 12 % was trapped in solids such as excrement. 28 % was oxidized to nitrate in the biofilter and another 7 % in pipes/ tank walls, and both were eventually removed as nitrogen gas.

Table 1. Mass balance of nitrogen, production and consumption rates in the closed recirculating aquaculture system for pejerrey\*<sup>1</sup>

Parameter	Feed	Feces			Ammonia	
	Consumed nitrogen* <sup>2</sup>	Sludge collector	Drum filter	RBC	Fluidized-bed filter	Others
Nitrogen (mg-N/h) * <sup>3</sup>	744±46	66±36	23±9	133±52	77±42	50±38
( % )	100	9	3	18	10	7

\*<sup>1</sup> Total body weight : 38.5kg, body weight : 109.1±37.8g (Mean±S.D., n=353) .

\*<sup>2</sup> Feed consumption rates : 10.5±0.65g/h (Mean±S.D., n=4), protein content in feed : 48%, nitrogen content in feed : 7.1% (Nippon Formula Feed MFG. EP NO.4 for juvenile Japanese flounder).

\*<sup>3</sup> Mean±S.D., n=4.

## Discussion

The nitrogen purification mechanism of this system

Major nitrogenous matter that it is excreted into the water by fish is ammonia, urea and solid excrement of undigested feed. Then, urea is degraded promptly in the water, where it becomes ammonia and carbon dioxide (Colt and Tchobanoglous, 1976). 47 % of ingested nitrogen was excreted and the remaining 53 % was assumed to taken up in fish and bacterial biomass.

The purification mechanism of this system was considered from the trapped amount of

nitrogen with each unit and the nitrification volume. Of the total nitrogen that was excreted by the fish, 60 % was oxidized to nitrate in the biofilter (38 % in the RBC, and 22 % in the fluidized bed filter) and another 14 % in pipes/ tank walls. In total 74 % was completely nitrified from ammonia to nitrate, and was eventually removed as nitrogen gas in the denitrification unit (Fig.3).

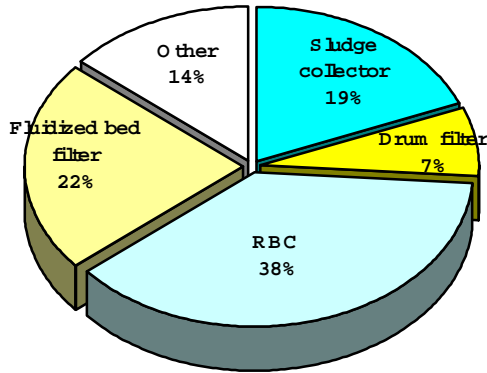


Fig. 3. The process rate of the nitrogen (excrement, ammonia nitrogen) that a fish excreted.

Furthermore, the remaining 26 % was trapped as solids (excrement) of which 19 % through the ECO-TRAP sludge collector and 7 % from the drum filter settling tank, and eliminated from the system. Uneaten feed and the solid excrement will not affect water quality immediately. However, after some time, decomposition of the organic and protein material will produce noxious substances. Therefore, the early elimination of uneaten feed and excrement is clearly shown to reduce the load to the biofilter.

### Denitrification

Tap water was used as the source for this experiment, and so there may have been few denitrification bacteria available in the system water. Also the system condition was kept completely aerobic with few organic deposits via stringent solids and mechanical filtration. In this environment anaerobic denitrification bacteria will not breed easily. (Dissolved oxygen concentration of the RBC influent,  $7.61 \pm 0.13 \text{ mg/l}$ ,  $n=4$ ) Therefore, until a denitrification unit was installed, it was considered that denitrification didn't occur. Though glucose was added after denitrification unit installation and small bacterial colonies appeared in the denitrification unit, denitrification did not proceed. Heterotrophic bacteria, other than denitrification bacteria multiplied with the addition of the glucose. Therefore, it was assumed that the preferential growth of denitrification bacteria was obstructed. Eventually, with the addition of methanol,  $\text{NO}_3\text{-N}$  concentration

achieved a 750 mg/l decline in the last 65 days, and pH and alkalinity increased greatly, requiring pH adjustment. It is already known that alkalinity rises from denitrification (Jeris and Owens, 1975). If the denitrification unit was installed since the culture initiation, the total consumption of pH adjusting chemicals can reduce and the denitrification unit can be miniaturized more.

The relation of the accumulation of the nitrate and the water interchange

The change of the nitrate nitrogen concentration when denitrification isn't done, and effect by the replacement water were modeled by using the excretory rate of the nitrogen obtained from this experiment. A calculation condition is shown in table 2. The nitrogen volume that it is excreted by a fish is shown as follows.

$$Wn_i = \frac{Wf_i}{1000} \times \frac{Fr_i \times Fcr_i \times Pf \times Nf \times Eer \times Ednr \times Nr}{100} \quad (1)$$

Table 2. Characteristics and value of calculation condition

Parameters		Value	Unit
Total body weight on day <i>i</i>	$Wf_i$	$Wf_1=10$ $Wf_{365}=5000$	kg
Feeding rate on day <i>i</i>	$Fr_i$	1	%
Food consumption rate on day <i>i</i>	$Fcr_i$	100	%
Protein content in feed	$Pf$	48	%
Nitrogen content in Protein	$Nf$	16	%
Excretory rate of the nitrogen	$Eer$	47	%
Dissolved nitrogen rate of the excrement	$Ednr$	74	%
Nitrification rate for one day	$Nr$	100	%
Weight of nitrate nitrogen on day <i>i</i>	$Wn_i$		g
Total volume of system water	$Tw$	130,000	l
Nitrogen concentration on day <i>i</i>	$Cni$		mg/l
Replacement water rate per day	$X$	0-10	%

When the replacement water rate was  $X$  per day, Nitrate nitrogen concentration on  $i$  day is shown at the next equation.



$$Cn_i = \left( \frac{Cn_{i-1}}{1000} \times Tw \times \frac{(100 - X)}{100} + Wn_i \right) \times Tw^{-1} \times 1000 \quad (2)$$

It was calculated about the case that it was reared in the commercial facilities size of the total volume of water 130 m<sup>3</sup> for 1 year. Fig.4 is the change of the nitrate nitrogen concentration when the replacement water rate was 0-10 % per day. A calculation is when the density of the fish per the tank increases linearly to 5 % from 0.01 % for 1 year, in a total system volume of 130 m<sup>3</sup> (Table 2). The feeding rate was 1, and all the feed containing 50 % protein containing rates was consumed. We postulate when 35 % of the nitrogen content of that was excreted as dissolved nitrogen such as ammonia. For this calculation we fixed the rates of nitrogen absorption and of excretion, though in reality these values vary according to species and growth stage.

When 10 % (13 m<sup>3</sup>/day) of the total system volume is replaced per day, nitrate nitrogen concentration reaches 100 mg/l for 1 year. In this case, water with the nitrate nitrogen concentration of 100 mg/l is discharged at a rate of 13 m<sup>3</sup> per day. With zero water exchange, nitrate nitrogen accumulates to a concentration of nearly 2000 mg/l after 1 year.

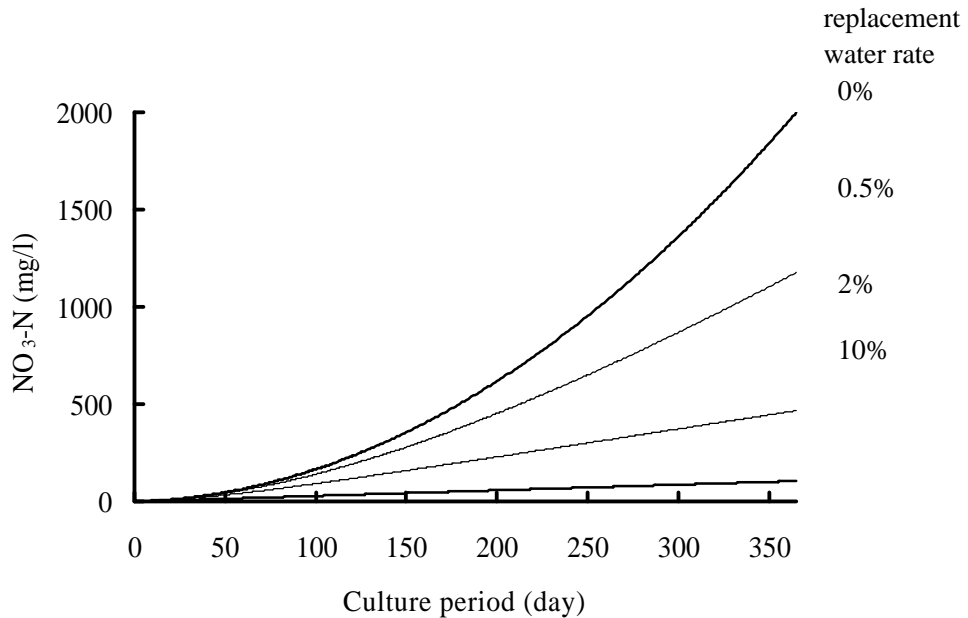


Fig. 4. The change of the nitrate nitrogen concentration in the culture water when the replacement water rate was 0-10 % per day. Calculated for the case where the total volume of water was 130 m<sup>3</sup> for 1 year.

The nitrate concentration of the culture water is greatly affected by the metabolic rate of the fish, and exchange rate of new water. And, the decrease of the nitrate concentration

becomes possible without water replacement if denitrification is done. Denitrification is considered necessary when closed recirculating aquaculture systems are used though there is little study of fish tolerance toward the nitrate. Current design practices should consider an acceptable nitrate nitrogen concentration, a water exchange rate based on site conditions and the denitrification filter sized accordingly.

### **Acknowledgement**

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# Comparisons of Four Commonly Used Aquatic Plants for Removing Nitrogen Nutrients in the Intensive Bioproduction Korean (IBK) Recirculating Aquaculture System

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## Introduction

In a high density recirculating aquaculture system, nitrate accumulation is unavoidable (Honda et al. 1993) because excreted ammonia from fish is oxidized to nitrates through nitrification process in the biofilter (Sawyer and McCarty 1978; Colt and Armstrong 1981). Though nitrate-nitrogen is not as toxic as ammonia or nitrite (Russo and Thurston 1991) it affects the pH of rearing water and is known to stress fish at high concentration (Hrubec et al. 1996).

There are a few methods to remove accumulated nitrates from recirculating aquaculture systems. Denitrification by anaerobic bacteria is one of the common methods for this but it needs anaerobic condition in the system and the water from denitrification chamber must be well aerated before flowing into the system. It also needs external carbon sources (Payne 1973) and pump or diversion from main flow pipe system. Increasing the water exchange rate with new water is another common method but it has some constraint if rearing water is either heated or cooled. The bigger the temperature difference between rearing water and new water the more energy is needed. Using aquatic plants or vegetable is another common method of denitrification. Dunigan et al. (1975) reported that *Eichhornia crassipes* absorbs nitrates and phosphate from the pond aquaculture system. This plant was also tested to reduce pollution level from the industrial effluent and river and stream (Lee 1993). Aquatic plants do not need much energy (Reed et al. 1988) for denitrification and they grow well under proper water temperature and light conditions. Hydroponics with vegetables or other valuable plants is not only pursuing denitrification in the system (Rakocy 1994) but also enhance the income of fish farmers (Watten and Busch 1984).

Four different aquatic plants, *Eichhornia crassipes*, *Hydrocotyle leucocephala*, *Hygrophila angustifolia*, and *Pistia stratiotes*, were originally introduced into Korea from

tropical countries for tropical fish aquaria and have been used for denitrification process in recirculating aquaculture systems by some farmers in Korea. However, denitrification capacity of these plants has not been evaluated. Therefore the removal capacity of ammonia, nitrite, and nitrate from recirculating aquaculture systems by these aquatic plants was compared.

## Materials and Method

In experiment 1, the absorbing capacities of total ammonia-N ( $\text{NH}_4\text{-N}$ ), nitrite-N ( $\text{NO}_2\text{-N}$ ), and nitrate-N ( $\text{NO}_3\text{-N}$ ) were tested in 200 L aquaria. Fifty liters of rearing water from an existing Intensive Bioproduction Korean (IBK) system (Kim and Jo 1998) fish farm was filled in each aquarium and 500 g each of *Pistia stratiotes*, *Hygrophila angustifolia*, *Eichhornia crassipes*, and *Hydrocotyle leucocephala*, was carefully washed, weighed and placed in the aquaria.  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$ , and  $\text{NO}_3\text{-N}$  in the water were measured at every 4 hours for 48-hours. The experiment was carried out under temperature conditions of  $30^\circ\text{-}38.5^\circ$  (September 12-14).

In experiment 2, the growth of the four plants was evaluated for 30 days in the IBK system and proximate analyses of the plants were made after the experiment. Each of 2.0 kg of the plants was planted in the wooden frameworks of  $2\text{ m}^2$  ( $2 \times 1\text{ m}$ ), which were installed at the top of biofilter of the system. This experiment was carried out from September 10, 1999 to October 9, 1999. Water temperature during the experimental period ranged from  $28^\circ$  to  $32^\circ$ .

After the growth experiments, sample of each plant was taken and made freeze dry for the evaluation of moisture content of live plant (AOAC 1995). The freeze-dried samples were used for proximate analyses. Crude protein, moisture and ash of freeze dried and powdered plants were analyzed by Association of Official Analytical Chemists (AOAC 1995) methods. Crude fat was determined using the Soxtec System 1046 after the sample was freeze-dried.  $\text{NH}_4\text{-N}$  was measured by Orion 720A (Orion Co., USA) and  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  were measured by DR2000 (HACH Co. USA). After the analysis, nitrogen removal capacity was calculated and compared with each other.

## Results and Discussion

The changes of concentrations of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  in aquarium water in experiment 1 are shown in Table 1. It was observed that *Pistia stratiotes* was most effective to reduce  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  in the water followed by *Eichhornia crassipes* and *Hygrophila angustifolia*, but *Hydrocotyle leucocephala* with negative results. The concentrations of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  in the aquarium water with *Pistia stratiotes* decreased from 2.3 ppm, 0.33 ppm, and 20.4 ppm to 0.4 ppm, 0.027 ppm, and 15.3 ppm, respectively in 48 hours.

For *Hydrocotyle leucocephala* the concentrations of  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  were rather increased with time. It seems to have been caused by the characteristics of this creeping long vine plant. The plant had to be cut into pieces to plant in the aquaria. From this process, some part of this plant seemed decayed, adding nutrients into water rather than removing them.

In experiment 2, *Pistia stratiotes* grew fastest in terms of total wet matter, but almost same growth with *Hygrophila angustifolia* in dry matter. *Eichhornia crassipes*, and *Hydrocotyle leucocephala* showed much inferior growth both in wet and dry matter among them (Table 2). The total weights increased 11.2, 7.4, 6.4, and 4.2 times initial biomass in 30 days, respectively.

Table 1. Absorption of inorganic nitrogen from the water by four aquatic plants in the aquarium in experiment 1 (30.1-38.5°C). The water was brought from an existing IBK system fish farm. (unit: mg/L in water).

Aquatic Plants	NH <sub>4</sub> -N			NO <sub>2</sub> -N			NO <sub>3</sub> -N		
	Initial	24h	48h	Initial	24h	48h	Initial	24h	48h
<i>Pistia stratiotes</i>	2.3	1.1	0.4	0.197	0.029	0.024	21.4	20.1	17.4
<i>Hygrophila angustifolia</i>	2.3	1.7	1.6	0.197	0.170	0.15	21.4	20.1	20.1
<i>Eichhornia crassipes</i>	2.3	1.4	0.6	0.197	0.057	0.029	21.4	20.8	17.9
<i>Hydrocotyle leucocephala</i>	2.3	1.7	2.3	0.197	2.91	7.8	21.4	28.4	62.8

Table 2. The growth performance of four aquatic plants at the top of filter bed of the IBK system for 30 days and proximate analysis of the plants in experiment 2.

Item	<i>Pistia stratiotes</i>	<i>Hygrophila angustifolia</i>	<i>Eichhornia crassipes</i>	<i>Hydrocotyle leucocephala</i>
Initial biomass (g)	2,000	2,000	2,000	2,000
Final biomass (g)	22,376	12,774	14,805	8,482
Final/Initial ratio	11.2	6.4	7.4	4.2
Biomass produced (g)	20,376	10,774	12,805	6,482
Moisture (%)	90.00	80.20	92.30	89.20
Dry Matter (DM) (%)	10.00	19.80	7.70	10.80
Biomass produced, DM (g)	2,038	2,133	986	700
Crude protein in DM (%)	4.61	2.62	4.75	4.85
Crude protein in WM (%)	0.46	0.52	0.37	0.52
Crude protein (g)	93.93	55.89	46.83	33.95
Nitrogen value (g)	15.03	8.94	7.49	5.43
Crude fat in DM (%)	22.44	15.40	15.48	22.86
Crude fat in WM (%)	2.24	3.05	1.19	2.47
Crude fat (g)	457.24	328.52	152.63	160.03
Crude ash in DM (%)	24.18	12.71	18.1	17.99
Crude ash in WM (%)	2.42	2.52	1.39	1.94
Crude ash (g)	492.69	271.14	178.46	125.94

DM: dry matter; WM: wet matter

In terms of total nitrogen (N) synthesized by the plants on an area of 2 m<sup>2</sup> for 30 days, *Pistia stratiotes*, with an amount of 15.03 g, was considerably superior to the other 3 plants, *Hygrophila angustifolia* (8.9 g), *Eichhornia crassipes* (7.49 g), and *Hydrocotyle leucocephala* (5.43 g). Thus the fastest growth rate of *Pistia stratiotes* in experiment 2 well agrees with the

fastest absorption of nutrients from water in experiment 1. Therefore *Pistia stratiotes* seems the best for denitrification purpose among the plants tested for the IBK system.

In the present experiment initial planting was so thin that the spatial potential to grow the plants was not fully utilized. Therefore if we had started the plant growing much intensely from the first, the production would have been more than double or triple the present results of 15 g nitrogen synthesis. Thus the amount of nitrogen synthesis by *Pistia stratiotes* would have been more than 30 to 45 g on an area of 2 m<sup>2</sup> for 30 days.

The amounts of crude fat and ash synthesized were also highest in *Pistia stratiotes* (457 g and 492 g, respectively) followed by *Hygrophila angustifolia* (328 g and 271 g), *Eichhornia crassipes* (152 g and 178 g) and *Hydrocotyle leucocephala* (160 and 125 g).

## Conclusion

Some aquatic plants such as *Eichhornia crassipes*, *Hydrocotyle leucocephala*, *Hygrophila angustifolia*, and *Pistia stratiotes*, are used for denitrification process in recirculating aquaculture systems by some fish farmers in Korea. The removal of NH<sub>4</sub>-N, NO<sub>2</sub>-N, and NO<sub>3</sub>-N from the water of IBK system by these four aquatic plants was compared. Weight gain of, and nitrogen synthesized by, these plants in the system were evaluated. *Pistia stratiotes* appeared much more effective than the other three plants both in terms of total synthesis and the removal of inorganic nitrogen nutrients from the water of IBK system.

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# Recirculation System Design; The KISS Concept

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Having installed over 80.000 m<sup>3</sup> of intensive culture pond volume Haogenplast Projects is a major fish farm constructor. Large efforts were made to develop cost-effective recirculation systems. Experience was gained with roundfish, flatfish and shrimp, both in seawater and fresh water. A wide variety of equipment and system set-ups were tested and evaluated. Finally this work is yielding various effective solutions. The key is the KISS rule; Keep It Simple, Stupid! The system concept is described that is now in use for culture of shrimp, Hybrid striped bass and Seabream.

The fish or shrimps are grown in D-ended raceways of 100-500 m<sup>2</sup>, 0.6-1.2 m. deep. A central baffle and paddlewheel aerators provide a one-directional current as well as O<sub>2</sub> addition and CO<sub>2</sub> stripping. The farm is in a greenhouse that is covered with transparent plastic (Tilapia), green plastic (Bass) or an insulating material (Flatfish). A special bottom outlet removes settled solids, while the main water treatment flow is taken via an overflow weir. Densities are kept modest (10-20 kg/m<sup>3</sup> for fish).

The main water treatment consists of a combination of a hydrocyclone / settler and beadfilter for solid removal and submerged aerated biofilters. Both components use "Haogenplast macaroni", small extruded tubes with a large surface area. Pumping is done by airlift pumps.

The solid catchers are periodically aerated for cleaning and the backwash water is directed to a covered denitrification pond. Here organics are digested, nitrate is turned to nitrogen gas and alkalinity is recovered. The effluent of the denitrification pond can be discharged or re-directed into the system. Small system adaptations are made if the cultured species requires so.

The combination of large water volumes, large water flows and an overdimensioned watertreatment unit assure a good and stable water quality. The strong dependence on aeration by blowers makes the system technically simple and highly reliable, even in seawater.

The system components are designed for one task but have dual purpose. Airlifts pump water but significantly contribute to gas exchange. Depending on the water quality they also function as foam fractionator. The beadfilter is designed for solid capture but gives a bonus by working as biofilter as well.

High energy efficiency is achieved by fine-tuning the airlift design and by operating paddlewheels and airlifts on-demand. This is achieved by an advanced



onitoring/control system that also regulates the temperature in the farm through regulating passive ventilation and by operating heat-reflective screens.

Technical data about the farm design, technical and biological performance will be presented.

# **The Japanese Aquaculture Market and Changes into the 21st Century**

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Despite this small island nation of 120 million population being the world's largest seafood importer, Japan's mariculture industry, by far the world's most mature, is suffering from the effects of the longest post WWII recession as well as structural and market obstacles to profitability. Producer morale is low and the outlook is poor. In this paper, I will describe briefly the historical development of the large industry with the intent that other nations can learn from Japan's mistakes and also I will cover some technologies that may help bring about recovery of this multi-billion dollar industry.

Current producers are plagued by low survival rates, lack of effective vaccines, unregulated access to antibiotics, extremely inefficient distribution, water pollution, toxic algae blooms, expensive feed and poor benthos quality. Farmers are leaving the aquaculture industry in droves as the red-ink piles up.

A large majority of farmed fish are shipped live in Japan due to the Japanese consumers' obsession with freshness required for enjoyable consumption of sushi and sashimi, uncooked forms of preparing fish. Approximately 95% of Japan's 70,000 ton production of red sea bream and about 65% of the 150,000 tons of *Seriola* are harvested and shipped to market in live holding trucks.

We are proposing to the Japanese producers and regulators several solutions to the current dilemma, some of which are already well underway. First disease needs to be prevented through an effective vaccine program, and many Japanese and foreign companies have registration applications underway. However, some laws in existence prevent for example long-term effective viral vaccines and we are lobbying for changes to these laws. Feed and feeding practice improvements will decrease feeding cost and decrease organic loading, providing synergistic improvements in environment and production efficiency. In concert with these production related improvements, much effort is being put into improving the distribution system with emphasis on live fish distribution technology, which the author has been concentrating on for the last two years. Current technology and that under development will be examined in detail.

# **Novel Live Seafood Shipping and Freshness Preservation Technology from Japan**

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