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# Performance of a closed recirculating system with foam separation, nitrification and denitrification units for intensive culture of eel: towards zero emission

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## Abstract

The development of a closed recirculating aquaculture system that does not discharge effluents would reduce a large amount of pollutant load on aquatic bodies. In this study, eel were reared in a closed recirculating system, which consisted of a rearing tank, a foam separation unit, a nitrification unit and a denitrification unit. The foam separation unit has an inhalation-type aerator and supplies air bubbles to the rearing water. The growth of eel, which were fed a commercial diet, was satisfactory, with gross weight increases of up three times in 3 months. The survival rate under the congested experimental conditions was 91%. The foam separation unit maintained oxygen saturation in the rearing water at about 80%. Furthermore, fine colloidal substances were absorbed on the stable foam formed from eel mucus and were removed from the rearing water by foam separation. Ammonia oxidation and the removal of suspended solids were accomplished rapidly and simultaneously in the nitrification unit. The ammonia concentration and turbidity were kept at less than 1.2 mg of N per litre and 2.5 units, respectively. When the denitrification process was operated, nitrate that accumulated in the rearing water (151 mg of N per litre) was reduced to 40 mg of N per litre. The sludge was easily recovered from the nitrification and denitrification tanks, and the components were found suitable as compost. Based on these results, the intensive aquaculture of freshwater fish such as eel can be achieved using a closed recirculating system without emission.

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*Keywords:* Aquaculture system; Recirculating; Eel; Foam separation; Zero emission

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## 1. Introduction

Recently, the technology of a recirculating aquaculture system with a high fish density has been developed (Bovendeur et al., 1987; Heinsbroek and Kamsta, 1990; van Rijn and Rivera, 1990; Ng et al., 1992; Knosche, 1994; Honda et al., 1994; Arbiv and van Rijn, 1995; van Rijn, 1996; Geiner and Timmous, 1998; Yoshino et al., 1999), and the remarkably high productivity and energy efficiency of such a system have become possible (Blancheton, 2000). However, the frequency of changing rearing water per day is from 5 to 100% in general, thereby having minimal effect on the reduction in pollutant load. In a recent study that examined the amount of pollution drained from inland aquaculture farms in Japan, it was found that the type of fish being cultivated made minimal difference. On average, the load per unit of cultured fish was 0.8 kg of N per tonne fish per day and 0.1 kg of P per tonne fish per day (Maruyama and Suzuki, 1998). This pollutant load corresponded to 73 persons per tonne fish assuming that the human nitrogen load is equivalent to 11 g N per person per day. The total fish production of inland aquaculture in 1999 in Japan is 63,000 t. Hence, pollutant discharge from aquaculture corresponds to the waste generated by five million persons. While the aquaculture industry supplies food and livelihood to many, it is likewise a bane to aquatic environments.

Worldwide, the development of a recycling system that brings environmental load close to zero is required today in most, if not all, industries. The aquaculture industry is likewise urged to convert to a new system that introduces the concept of zero emission, and this requires the development of a closed recirculating system using innovative technology for fish production. In many cases, however, some closed recirculation systems require the drainage of a certain percentage of rearing water into the environment. If rearing water is drained without appropriate processing, the high nitrogen and phosphorus loads contained in the drainage can pollute the environment, even if the frequency of change is very low. In addition, the sludge and water used to wash filter media are also directly discharged in many cases. The characteristics of an economical and environmentally friendly zero-emission system are as follows: water use is minimised, drainage water is purified to the same level as raw water and sludge is further utilised as fertilizer. The following contaminants produced during fish culture should be made as close to zero as possible: degradable organic substances, nitrogen and phosphorus. Currently, zero-emission aquaculture system in an actual aquaculture farm has not yet been developed. However, if intensive fish culture in a perfectly closed recirculating system becomes technically possible, the development of zero-emission systems can be realised.

With the combined aim of increased fish production and reduced nutrient load in aquatic environments, we have advanced the development of a zero-emission system composed of foam separation, nitrification and denitrification units. Culture trials of Japanese flounder in seawater (Maruyama et al., 1998; Suzuki et al., 2000) and eel in fresh water (Suzuki et al., 1999) were carried out using this almost perfectly closed system. The survival rate was very high, that is, more than 3 months despite the high fish density. The advantage of this system is that it is equipped with an effective foam separation unit as part of its main purification process. Oxygen supply, removal of suspended substances and deaeration can be achieved simultaneously by the foam separation process (Maruyama et al., 1991, 1996).

An ideal aquaculture system is one which purifies the rearing water while obtaining high biomass productivity. In this study, eel productivity, function of each water treatment process, sludge recovery and load reduction were examined.

## 2. Materials and methods

### 2.1. System description

A closed recirculating system with foam separation, nitrification and denitrification units is shown in Fig. 1. This system consisted of a fish-rearing tank ( $0.5 \text{ m}^3$ ; water volume  $0.43 \text{ m}^3$ ; water surface area  $1.0 \text{ m}^2$ ), a foam separation tank ( $0.25 \text{ m}^3$ ) equipped with an inhalation-type aerator (200 V, 0.2 kW), a nitrification tank ( $0.16 \text{ m}^3$ ) and a denitrification tank ( $0.21 \text{ m}^3$ ). The total amount of water in this system was  $1.05 \text{ m}^3$ . A heater (100 V, 1 kW) and a pH control pump (Iwaki Co., EH/W-PH, 5% sodium hydrogen carbonate solution) for adjusting the conditions of the rearing water ( $28^\circ \text{C}$  and pH 7.5) were set in the foam separation tank and a water conditioner was set on the recirculating pipe. First, tap water was introduced to the system and one cycle was carried out for 15 min at 56 l/min. The rearing water was transported to the foam separation tank by a circulating pump, and oxygen supply and foam separation processing were simultaneously carried out using this unit. The rearing water was then introduced into the nitrification tank with an up-flow style, and the treated rearing water was returned to the rearing tank. Water temperature, pH, electric conductivity (EC) and dissolved oxygen concentration of the rearing water were continuously monitored using a water quality monitoring system (Horiba Co., WP-100).

The main core of this system was the foam separation unit (Fig. 2), which was equipped with an inhalation-type aerator (Fig. 3, TAS Environmental Engineering Co., Japan). By rotating the impeller, negative pressure is generated at the back of the impeller, and air is

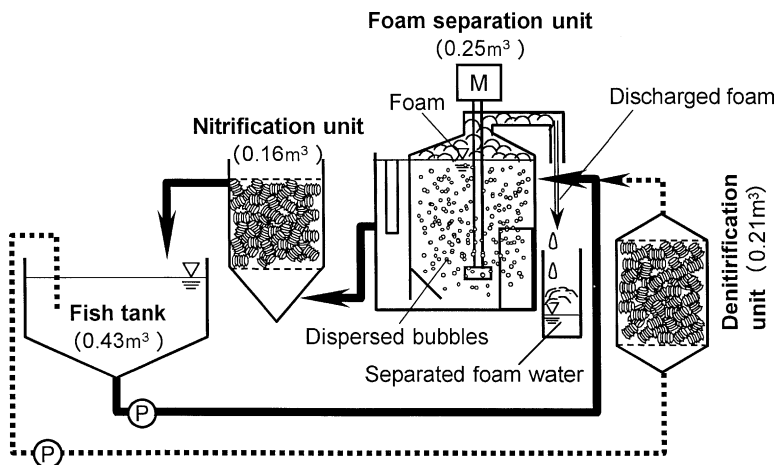


Fig. 1. Schematic diagram (not to scale) of the closed recirculating system with foam separation, nitrification and denitrification units.

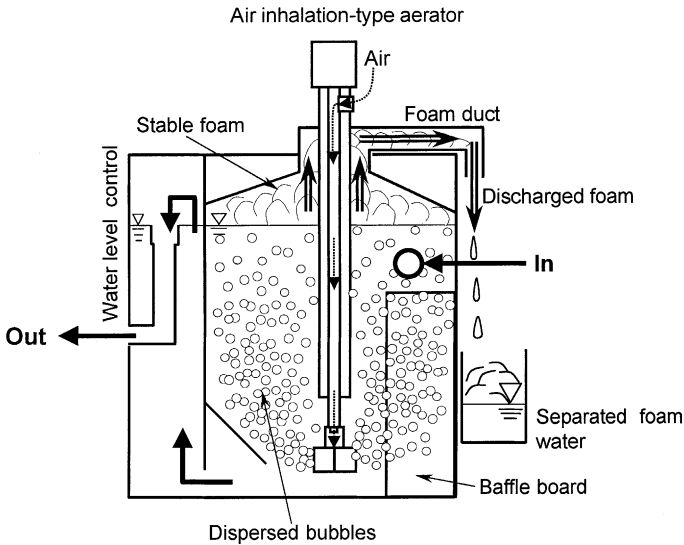


Fig. 2. Schematic diagram of the foam separation unit.

drawn from water in the shaft tube that connects to the outside environment (Fig. 3). Air is sheared with a blade immersed in water and numerous bubbles are extensively dispersed in water. Surface-active materials in the rearing water adsorb on bubbles, and the bubbles are carried to the water surface. Then, foam generates on the water surface. The foam generated

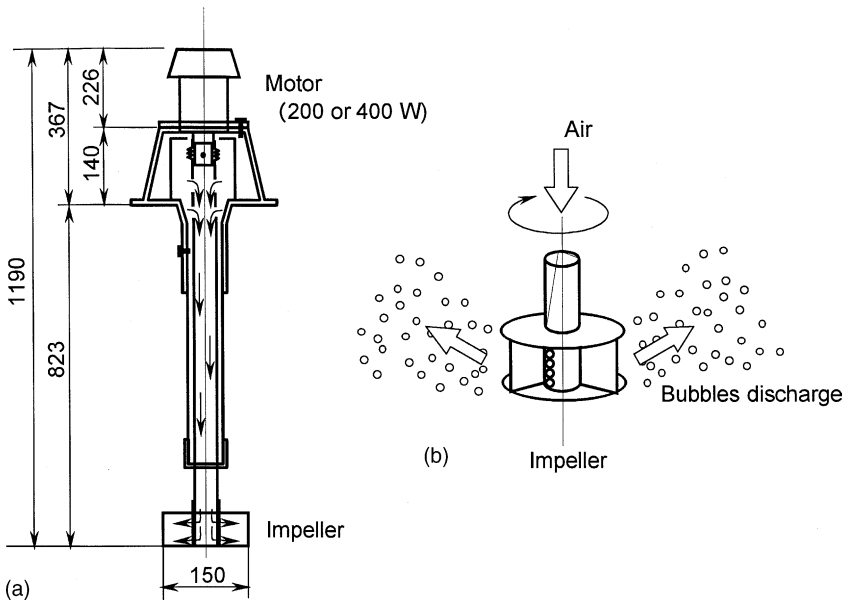


Fig. 3. Schematic diagram of an air inhalation-type aerator. (a) The whole shape (unit: mm), (b) the impeller shape.

continuously is spontaneously removed from the foam duct placed at the upper part of the tank equipped with air exhaust (Fig. 2). Furthermore, air bubbles were vigorously mixed in water and oxygen was efficiently dissolved in the rearing water until it passes through the foam separation unit.

A cylindrical medium made of polyethylene (Furukawa Electrician Industry Co., 14 mm diameter, 11 mm inside diameter, 14 mm length, 0.93 specific gravity) was used to fill the nitrification tank up to the 0.16 m<sup>3</sup> (surface area 93 m<sup>2</sup>) mark. Nitrifying bacteria were immobilised onto the medium prior to the fish-rearing experiment.

In the denitrification process, a portion of the rearing water was made to flow into the denitrification tank using another line via a circulating pump. The same medium as that used in the nitrification process was used as the denitrification medium. The methanol dose tube was established at the midpoint of the inflow line to the denitrification tank and methanol was continuously injected by a metering pump (Iwaki Co., EH-B15) with an appropriate amount of methanol corresponding to three times the concentration of nitrate nitrogen in the rearing water (Suzuki and Maruyama, 1999). Then, the mixture of rearing water and methanol was introduced into the denitrification tank. Methanol injection was adjusted taking into account the nitrate concentration in the rearing water every week. The treated water that passed through the denitrification tank was returned to the foam separation tank.

The above system is considered an almost perfectly closed system because water is added only to replace that which is lost to evaporation and foam generation.

## 2.2. Fish rearing

Larval eel *Anguilla japonica* (total gross weight 3.3 kg, 163 tails, about 20 g/tail) were placed in the rearing tank. In the initial part of the experiment, the proportion of total eel weight to water quantity in the rearing tank was 0.8%. Throughout the rearing experiment, the eel were fed a commercial diet (Chubu Shiryo Co., Japan, 48% protein) daily except Sundays. In the initial stage, 100 g of the feed was given once daily every 10 a.m. This became twice daily (10 a.m. and 6 p.m., feed 200 g) when the baiting became active. Eel were cultured for 104 days.

## 2.3. Procedure for sludge recovery

After the water supply to the nitrification tank was stopped by a by-pass pipe, the sludge that accumulated in the nitrification tank was drained from the bottom and then transferred to another tank. All medium was also removed from the denitrification tank and washed with sludge water. After washing, the media were returned to the nitrification tank and the sludge was allowed to settle for 2 h. The supernatant was returned to the system and fish rearing was continued in the usual way. The concentrated sludge was frozen until analyses. At the end of the experiment, the denitrification tank was also washed in the same way.

## 2.4. Analytical methods

To determine the quality of rearing water, a sample was collected daily (except Sundays) from the rearing tank before feeding. Dissolved oxygen (DO), turbidity as kaolin

standard (Mitsubishi Kagaku Co., SEP-PT-706D), total organic carbon (TOC, Shimadzu Co., TOC-5000), color as cobalt platinum standard, absorbance at 260 nm (E260, Shimadzu Co., UV-2200), ammonium ( $\text{NH}_4\text{-N}$ , HACH Co., DR-2000), nitrate ( $\text{NO}_3\text{-N}$ , HACH Co., DR-2000), nitrite ( $\text{NO}_2\text{-N}$ , HACH Co., DR-2000), total nitrogen (T-N), phosphate ( $\text{PO}_4\text{-P}$ ) and total phosphorus (T-P) were analysed. The standard platinum–cobalt method of measuring color was used, in which the unit of color is that produced by 1 mg Pt per l in the form of chloroplatinate ion. The collapsed-foam water samples were also obtained, and TOC, color, E260, suspended solids (SS), T-N and T-P were analysed. The analytical methods followed that of the Japanese Industrial Standard (JIS K 0102) or HACH Co. analytical manual.

Carbon and nitrogen in the solid samples, such as feed, fish tissue and dried sludge, were analysed using a CHN coder (analysed by Shimadzu Technoresearch Co., Japan). Phosphorus in solid matter was decomposed in a mixture of perchloric acid and nitric acid, and analysed in the same way as T-P in rearing water.

In order to isolate skin mucus glycoprotein from the foam water, solid ammonium sulfate was added up to 75% saturation to the separated foam water (total volume 270 ml collected over 24 h from the foam separation tank) and the solution was allowed to stand overnight at 5 °C. The precipitate obtained was dissolved in 50 mM Tris–HCl buffer (pH 8.5) containing 0.1 M NaCl, and the viscous solution (20 ml) was applied to a Sepharose CL-4B column ( $2.6 \times 110$  cm), previously equilibrated with the same buffer. Sialic acid in the fractions was analysed by the method of Uchida et al. (1977). Sialic acid-containing fractions were pooled and dialysed against distilled water. The dialysate was then lyophilised to obtain 38.0 mg of skin mucus glycoprotein.

### 3. Results and discussion

#### 3.1. Survival and growth of eel

The survival rate, eel growth and feed quantity during the experimental period (104 days) are shown in Fig. 4. The survival rate was 91% by the end of the study period and mortality was mainly due to a power outage and a water conditioner problem. Throughout the rearing period, the eel fed actively and their total weight increased over time. The total feed consumed by the end of the study was 11.6 kg, and the increase in eel weight was 7.7 kg. The feed conversion ratio was 67%, which is equal to that obtained by culture farms in Japan. The fish density in the rearing tank became 2.6%, which is about four times higher than that of conventional eel culture farms in Japan (about 0.6%). The additional amount of tap water to replace water loss through evaporation was 460 l. The total amount of water (rearing water, separated foam water, added tap water) used during the rearing period was 1535 l. In this system, 200 l of water was used in producing 1 kg of eel.

A histogram showing the number of eel at given sizes at the end of the experiment is shown in Fig. 5a. The number of individuals that grew over 100 g were 46, which corresponded to about 1/3 of the population. This confirmed that the gross weight of eel increased over three times in about 100 days under a closed recirculating condition. Furthermore, the rearing was continued after the experimental period, except for eel under 25 g/tail and over 150 g/tail.

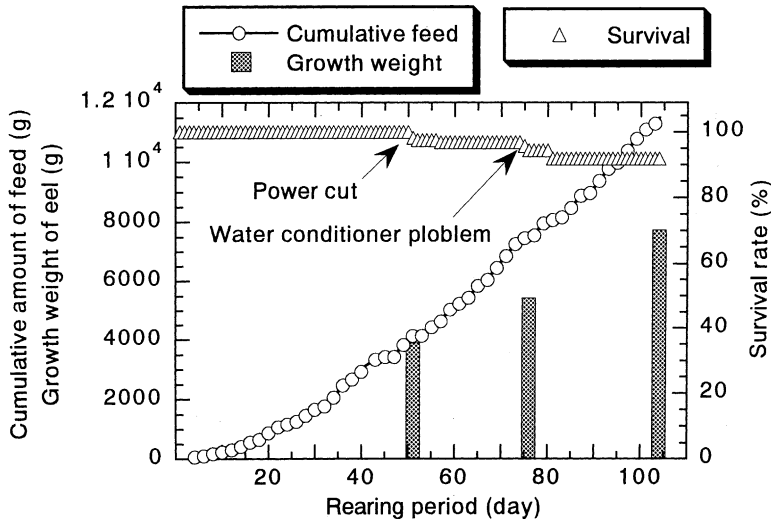


Fig. 4. Cumulative amount of feed, eel growth and survival rate during the rearing period.

In this extension period, the analyses of rearing water were not carried out. After 8 months (244 days), the gross weight reached 33 kg (in a rearing fish density of 7.6%), and about 90% of the eel attained commercial size (over 150 g/tail).

### 3.2. Quality of rearing water

#### 3.2.1. Dissolved oxygen

The changes in DO of the rearing water are shown in Fig. 6. The oxygen saturation percentage was kept above approximately 80% throughout the experimental period. The maximum and minimum DO concentrations were 7.16 mg/l and 3.74 mg/l, respectively, with a mean of 5.81 mg/l. The DO concentration decreased to 3.74 mg/l because water temperature increased, thereby lowering the DO saturation value, when the water conditioner momentarily encountered problems. While this system does not provide an extraneous source of oxygen except for the aerator in the foam separation tank, a high DO concentration was properly maintained in the rearing water.

#### 3.2.2. Turbidity, color and E260

The changes in turbidity, color and E260 of the rearing water are shown in Fig. 7. The turbidity of the rearing water was maintained in the range of 1–2 units, whereby almost no suspended substances could be observed. The turbidity standard for tap water in Japan is 2 units. However, the rearing water turned yellowish brown as the experiment progressed, and color and E260 gradually increased. The color determined by the platinum–cobalt method is useful in measuring the color of potable water and water whose color is due to naturally occurring materials. The color units reached 100° by the end of the study. E260 of the rearing water showed a very high correlation with TOC (Fig. 8). Therefore, the yellowish brown material that accumulated seemed to be slightly decomposed organic substances such as

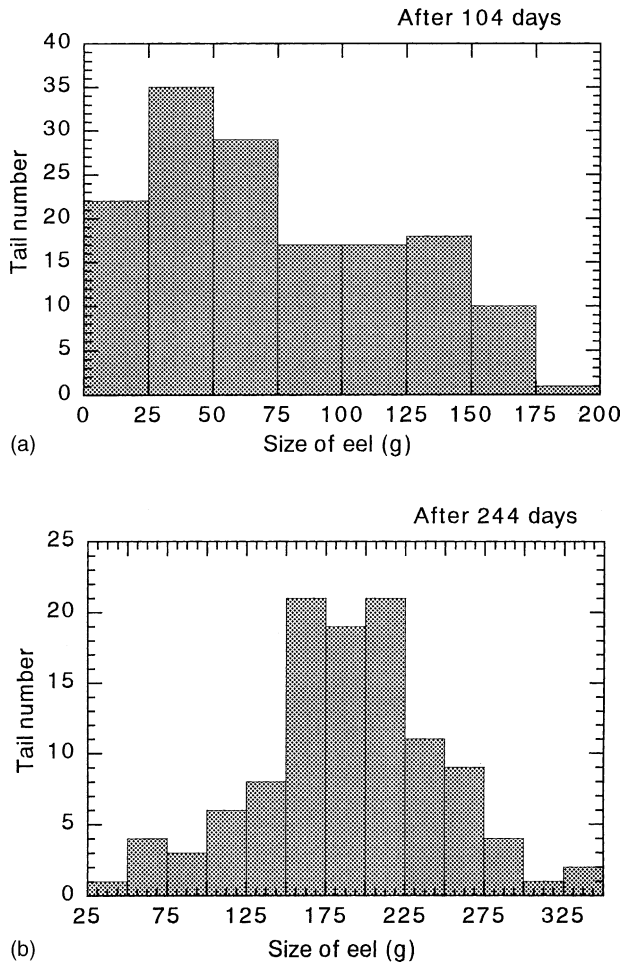


Fig. 5. The histogram of rearing eel during the rearing period.

humic (Tambo and Kamei, 1998). In this experiment, the accumulation of the yellowish brown material did not interfere with eel growth. Moreover, it is easy to remove the brown material via activated carbon adsorption (Suzuki et al., 2000) once the water color interferes with fish feeding or observation.

### 3.2.3. Nitrogen

The changes in the concentrations of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$  and T-N in the rearing water are shown in Fig. 9. While there was a slight increase in  $\text{NH}_4\text{-N}$  concentration, it was low at 1.2 mg of N per liter at the end of experiment. The  $\text{NH}_4\text{-N}$  concentration was kept low throughout the study period. The  $\text{NO}_2\text{-N}$  concentration was maintained at a very low level for 104 days. There was an increase in  $\text{NO}_2\text{-N}$  concentration on the 57th day because the sludge supernatant that was used in washing the nitrification tank was returned to the



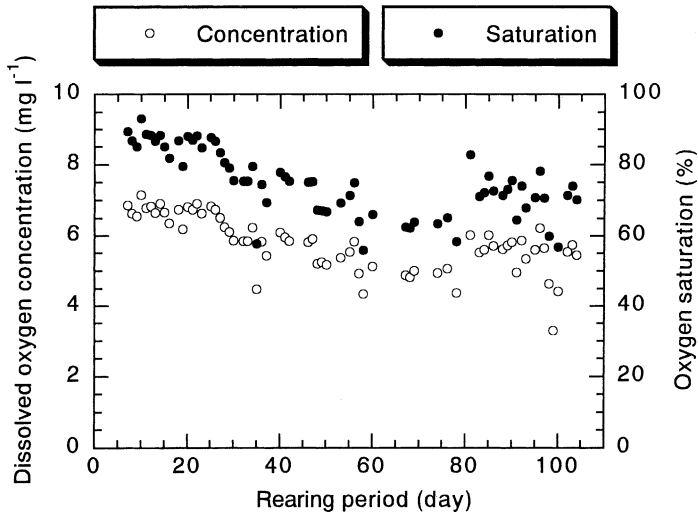


Fig. 6. Dissolved oxygen level in the rearing water.

system. However, the concentration immediately returned to a low level, indicating that the system's nitrification process functions well. In contrast,  $\text{NO}_3\text{-N}$  was formed via  $\text{NH}_4\text{-N}$  oxidation in the absence of denitrification, and  $\text{NO}_3\text{-N}$  steadily accumulated in the rearing water. However, when the denitrification process was initiated on the 42nd day,  $\text{NO}_3\text{-N}$  concentration began to decrease after about 1 week, and was reduced to 40.8 mg of N per liter by the end of the study. Organic nitrogen accounted for the difference in concentration between T-N and  $\text{NO}_3\text{-N}$ . The amount of injected methanol was higher at about 20% than

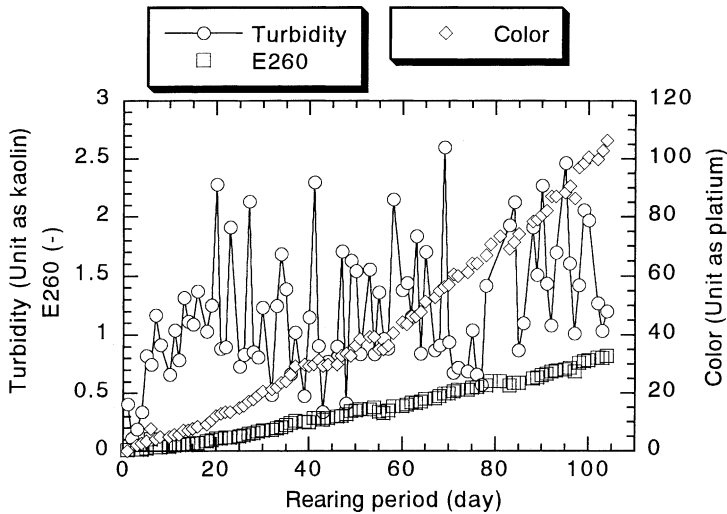


Fig. 7. Turbidity, E260 absorbance and color in the rearing water during the rearing period.

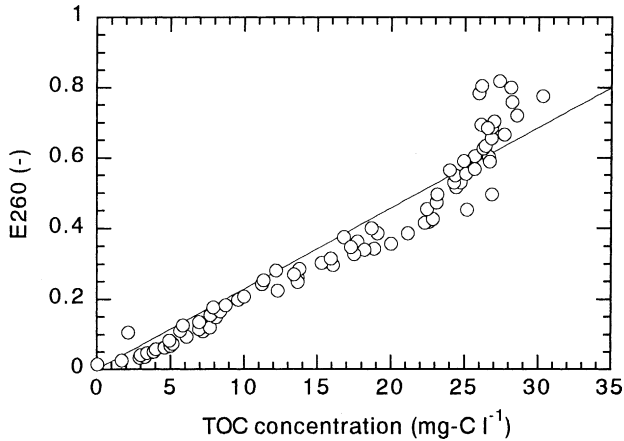


Fig. 8. Relationship between E260 absorbance and TOC concentration in the rearing water.

the stoichiometric methanol quantity needs for denitrification reaction, so that the quantity of residual methanol would be small. Actually, the interference effect of eel growth could not be observed with denitrification. It seemed that residual methanol that passed through the denitrification tank was biodegraded under aerobic condition until circulation in the system.

In the period without denitrification, the cumulative amount of feed intake ( $x$ ) and the amount of  $\text{NO}_3\text{-N}$  ( $y$ ) in the system showed a good correlation ( $y = 0.0342x$ ,  $r = 0.984$ ; Fig. 10). For example, when 100 g of feed was given to the eel, 3.42 g of  $\text{NO}_3\text{-N}$  accumulated in the rearing water. If the denitrification process did not operate throughout the rearing

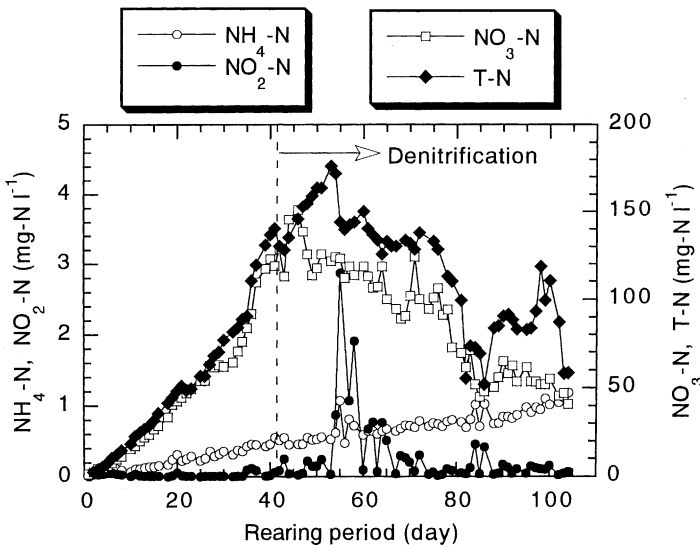


Fig. 9. Concentration of nitrogen compounds in the rearing water during the rearing period.

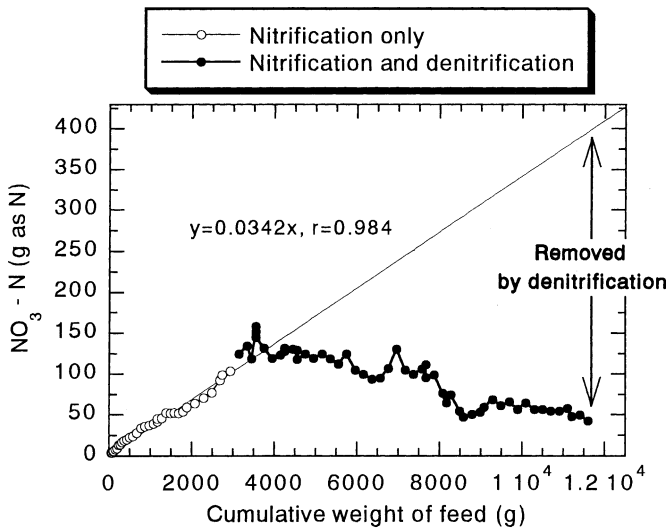


Fig. 10. Relationship of nitrate quantity as nitrogen to the cumulative amount of feed in the closed recirculating system.

period, 397 g N (total feed 11,600 g) in the form of  $\text{NO}_3\text{-N}$  would have accumulated in the rearing water. However, because of denitrification, the nitrate residue in rearing water was only 43 g N at the end of the study period. This showed that denitrification removed 90% of the  $\text{NO}_3\text{-N}$  that could have accumulated in the rearing water.

### 3.2.4. Phosphorus

The changes in phosphate and T-P in the rearing water are shown in Fig. 11. Most of the phosphorous in the rearing water was phosphate because it accumulated only in the absence of a phosphate removal process in this system. After eel production, phosphate in

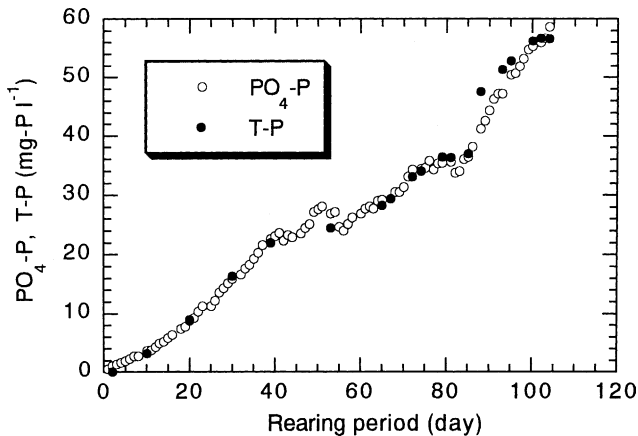


Fig. 11. Concentrations of phosphorus compounds in the rearing water during the rearing period.

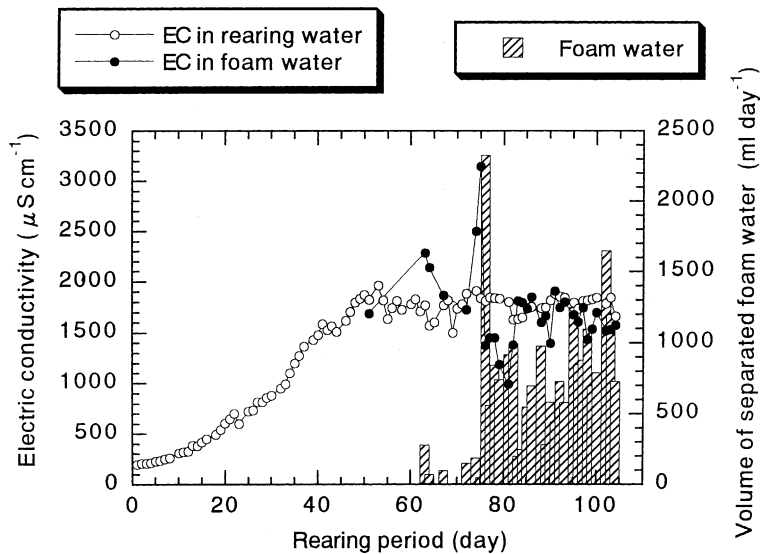


Fig. 12. Changes in electric conductivity in the rearing water and the foam water, and generated volume of the foam water during the rearing period.

the rearing water should be removed using appropriate processing methods such as chemical precipitation.

### 3.3. Characteristics of foam separation process

#### 3.3.1. Foam generation

The amount of foam water discharged and the changes in EC in the rearing and foam water samples are shown in Fig. 12. The discharged foam water was observed on the 51st day of the study period and foam water was continuously generated from the 63rd day to the end of the experiment. The total amount of discharged foam water was 25.3 l (2.5% of the total volume in this system), and the average quantity of water discharged per day was 589 ml ( $n = 43$ ). It has been reported that foam generation of fish mucus is dependent on the concentrations of mucus and coexisting solvent ions (Suzuki and Maruyama, 2000). Therefore, foam generation was considered to be related to the concentration of surface-active materials, such as fish mucus, and EC in rearing water.

In studies on the function of secretions from fish skin, the isolation of mucus glycoprotein from fish skin was performed using a Sepharose CL-4B column (Asakawa et al., 1989; Sumi et al., 1997). In eel skin mucus glycoprotein, a large number of disaccharide units, *N*-acetylneuraminy( $\alpha$ , 2  $\rightarrow$  6)*N*-acetylgalactosamine, link to the hydroxyl groups of threonine and/or serine residues in the polypeptide core with a molecular weight of more than 500 kDa (Asakawa, 1983). As shown in Fig. 13, mucus glycoprotein in the foam water was eluted at the void volume of the Sepharose CL-4B column in the same manner as described above. This reveals that the substance that contributed to the suspended solids in foam generation is mucus glycoprotein secreted from the skin of eel cultured in the rearing tank.

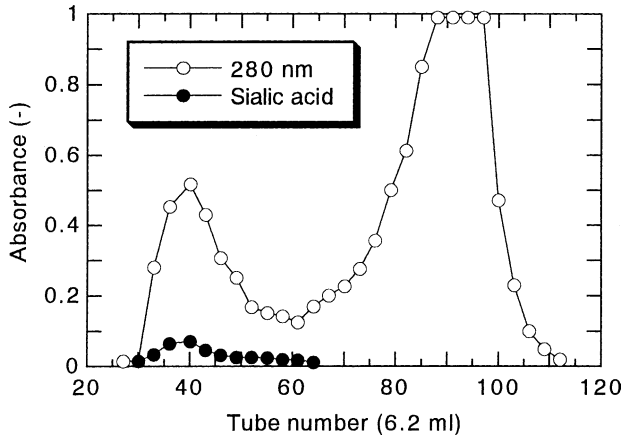


Fig. 13. Sepharose CL-4B chromatography of eel skin mucus glycoprotein obtained from the foam water.

3.3.2. Removal of suspended solids and color components

The suspended solids were significantly concentrated in the separated foam water and the turbidity of the separated foam water was one to two orders of magnitude higher than that of the rearing water (Fig. 14). The SS concentration in foam water changed irregularly, and varied from 400 to 4000 mg/l, making it necessary to remove SS from the system by a foam separation process. SS was not analysed in the rearing water since only small amounts

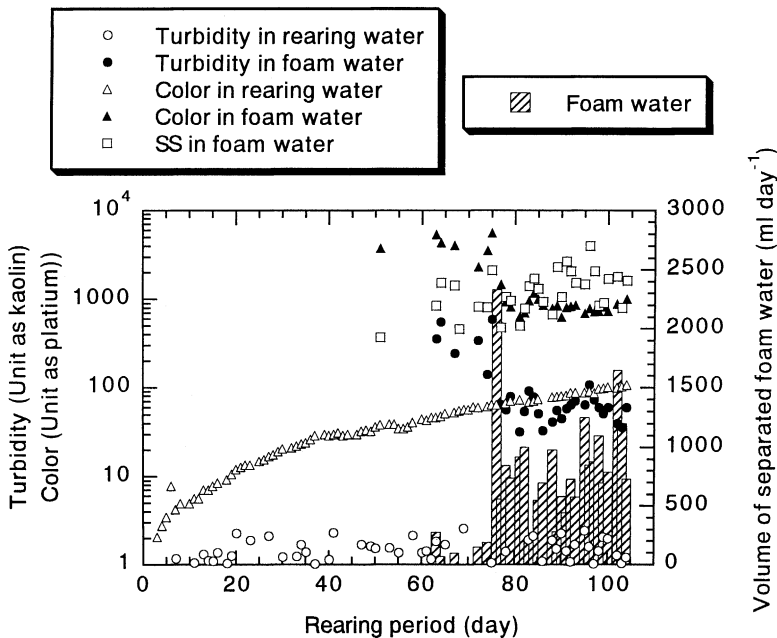


Fig. 14. Comparison of the rearing water with the foam water for suspended and colloidal matters.

were observed, and that the turbidity of the rearing water was retained between 0.5 and 2.5 units. Generally, SS and turbidity show a linear relation and the ratio of SS to turbidity is approximately 1. Although, in this study, the turbidity of the rearing water was 2.5 units or less even at maximum, it is assumed that SS is 3 mg/l or less throughout the experiment period. Moreover, a brown material was significantly concentrated in the foam water. The color unit of the foam water was 100–1000 times higher than that of the rearing water (Fig. 14). The foam separation process was able to remove the color components, which are difficult to remove by biological treatment or physical filtration. While an analysis of the effect of bacterial removal was not undertaken in this study, it has been reported that bacteria are concentrated and suspended in foam water (Maruyama et al., 1991, 1996; Suzuki et al., 2000).

### 3.3.3. Removal of nitrogen and phosphorus

The T-N concentration in the foam water was several times higher than that in the rearing water. Since most of the nitrogen components were NO<sub>3</sub>-N, highly efficient removal was not attained by foam separation. The T-P concentration in the foam water, which ranged from 150 to 350 mg P per liter, was also higher than that in the rearing water. The removal efficiency of phosphorus by foam separation was higher than that of nitrogen.

### 3.4. Recovery and utilisation of sludge

Suspended solids accumulated in the nitrification tank as the rearing period progressed. Therefore, in order to prevent plugging of the nitrification tank, cleaning of the medium and recovery of the sludge were carried out on the 54th day of the experiment. By the end of the study, the sludge that accumulated in the nitrification and denitrification tanks were also recovered. The total amount of sludge was 2014 g dry weight. About 96% of the total sludge accumulated in the nitrification tank. The polypropylene cylindrical medium was very light, such that taking them out from the nitrification tank was very easy. The sludge immediately separated from the media after washing and then settled down. The sedimentation velocity of the sludge was rapid and the sludge settled down in 10 min. This proved that the sludge could be quickly separated into solid and liquid components.

The components of the recovered sludge from the nitrification tank on the end were analysed and the C, N and P contents as dry weight were 17.4, 2.9 and 12%, respectively, and the calculated C/N ratio was 5.9. The C/N ratio and N content satisfied the organic fertilizer composition recommended by the Japan Sewage Works Association (1994). Moreover, the phosphorus content of the sludge was also very high, making it possible to use the recovered sludge as a good compost material.

### 3.5. Mass balances

The total amount of feed was 10,717 g dry weight (dried at 110 °C) during the rearing experiment for 104 days. The N and P contents in the feed were 8.0 and 2.9%, respectively. The eel production was 2669 g dry weight. The N and P contents in the fish body were 8.2 and 2.0%, respectively. The total N and P contents in the feed were considered as 100%, and the mass balances of this system are shown in Fig. 15.

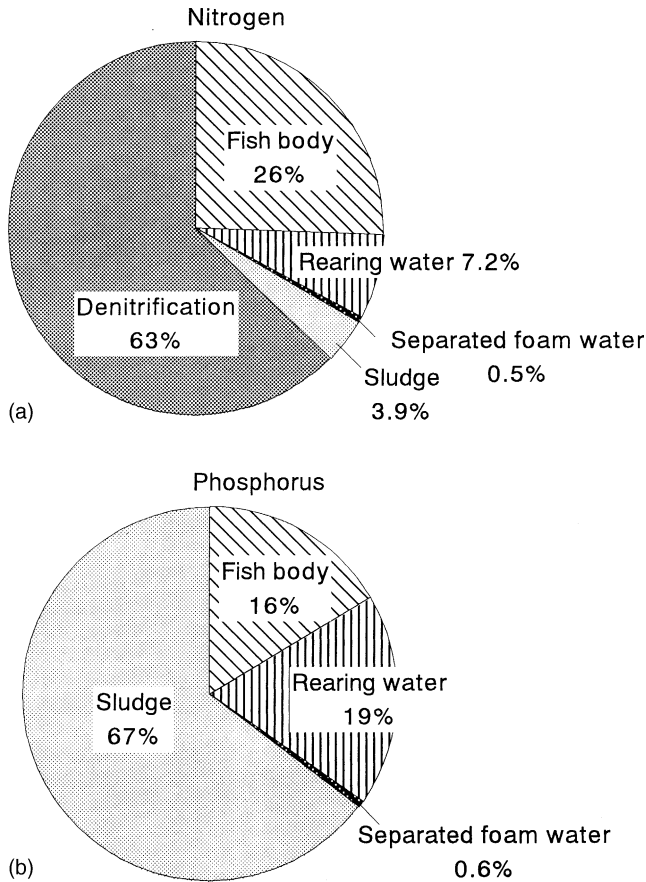


Fig. 15. Mass balances of nitrogen (a) and phosphorus (b) in the closed recirculating system.

In the case of total nitrogen, 26% was utilised for eel growth, 7.2% was accumulated in the rearing water as nitrate and organic nitrogen, 0.5% was removed by foam separation and 3.9% was accumulated in the nitrification and denitrification tanks as sediment (Fig. 15a). Regarding mass balances in the culture, the assimilation of nitrogen in the fish body varied from 25 to 35% of the total nitrogen input without regarding the difference in fish species (Folke and Kautsky, 1989; Hall et al., 1992; Maruyama and Suzuki, 1998; Skjølstrup et al., 1998; Suzuki et al., 1999). These results agree well with this study. Although the nitrogen content in the sludge was very small for the total nitrogen input, the close values obtained in this study were reported in other culture systems. The nitrogen contents in the sludge were 6% in the recirculation system for rainbow trout (Skjølstrup et al., 1998) and 5% in the flowing system for carp (Maruyama and Suzuki, 1998). Almost all the nitrogen that must be treated in this system was present as a dissolved fraction. In this study, the remaining 63% of nitrogen in the system was removed as nitrogen gas by denitrification. Denitrification could have removed the residual nitrate in the rearing water if

the operation was continued for a few days after the fish was harvested. Since the recovered sludge can be utilised as fertilizer, the zero emission of nitrogen is almost possible in this system.

In the case of phosphorus, 17% was utilised for eel growth, 19% was accumulated in the rearing water, 0.6% was removed by foam separation and 67% was accumulated in the nitrification and denitrification tanks as sediment (Fig. 15b). Because of analytical error, the total percentage exceeded 100%. The percentage of phosphorus in the eel was lower than that of nitrogen. The decrease in the phosphorus content in the fish (17–28% of the total phosphorus input) was observed in other previous reports (Folke and Kautsky, 1989; Holby and Hall, 1991; Maruyama and Suzuki, 1998; Suzuki et al., 1999). On the other hand, the proportion of phosphorus in the sludge remarkably increased further than that of nitrogen. Phosphorus was loaded as suspended substances and accumulated in the sludge in the nitrification tank. Sludge recovery and effective sludge utilisation are important in order to achieve the zero emission of phosphorus.

#### 4. Conclusions

Our proposed system achieved intensive eel culture in a perfectly closed cycle for more than 3 months. Eel growth was satisfactory and gross weight tripled during the study period, with a survival rate of 91%. In order to produce 1 kg of eel, 200 l of water was used. Oxygen was efficiently supplied to the rearing water by a foam separation unit and oxygen saturation was maintained at 80% throughout the experiment. Simultaneously, the foam separation process removed the brown colloidal substances generated by fish mucus. The nitrification tank removed suspended solids and likewise rapidly nitrified  $\text{NH}_4\text{-N}$ . While nitrate accumulated in the rearing water in the absence of denitrification, after it was initiated on the 42nd day, effectively removed  $\text{NO}_3\text{-N}$  and reduced it to 40 mg of N per litre at the end of the study. About 90% of the total nitrogen in the system was removed by denitrification. Sludge was easily recovered from the nitrification and denitrification tanks and proved to be suitable as compost material.

This system has a high application potential as a novel aquaculture technology that aims at zero emission. The next step is to evaluate its economic feasibility while considering fish production as well as initial and running costs.

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