

Effects of Cultured Fish Feces on Algae Growth

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ABSTRACT

In order to understand the role of fish feces in material cycling in aquaculture grounds, the effects of cultured fish feces to algal growth rates were examined by feeding probiotic supplements under laboratory conditions. Four 1000-L round-shaped plastic tanks were employed for culturing red sea bream, *Pagrus major* (average BL=12.5 cm). Twenty fish were reared in each tank with a running water system. The fish in tanks A, B and C were fed on moist pellets with 1% of *Ulva pertusa* fragments, 0.1% of yogurts and 0.5% of *U. pertusa* fragments and 0.05% of yogurts, respectively. The fish in tank D were fed on dry pellets alone. The feces were collected once a day and fermented by a rotator for 14 days. Algal growth potential (AGP) was determined for the culture of *Nannochloropsis oculata*. The algae were inoculated by fecal medium which was extracted after fermentation in an incubator. The temperature and light conditions in the experiments were adjusted to 23°C, 7500 lux and 12L:12D. The feces extracts collected from tanks A, B and C showed higher AGP in each trial. The feces collected from tank D presented always lower AGP. The highest population density of *N. oculata*, 34.8×10^6 cells/ml, was observed in tank A which was fed moist pellets with 1% of *U. pertusa* fragments.

INTRODUCTION

The conversion rates of food to fish are well studied in aquaculture. From the viewpoint of aquaculture ecology, however, bioconversion of fish to feces, or bioconversion of feces to algae is as important as the bioconversion of food to fish. In order to understand the role of fish feces in aquaculture farms, the effects of cultured fish feces to algal growth rates were examined by feeding probiotic supplements in laboratory conditions. In the present experiments, effects of cultured fish feces to algal growth were examined.

MATERIALS AND METHODS

The fish culture experiments were conducted in the Azumacho Fish Seedling Center in November 1994. Four 1000 L round-shaped plastic tanks, A, B, C and D, were employed for culture of red sea bream, *Pagrus major* (average BL=12.5 cm). Twenty fish were reared in each tank with a running water supply system. The fish in tanks A, B, and C were fed on moist pellets with 1% of *Ulva pertusa* fragments, 0.1% of yogurts, and 0.5% of *U. pertusa* fragments and 0.05% of yogurts, respectively. The fish in tank D were fed on dry pellets alone. The sterile *U. pertusa* were cultured in the fish farm around the center and were minced by the specially designed device for moist pellets. The yogurts containing multispecies of the lactate-fermenting bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, were obtained daily from a local

food store. The fish were satiated in the morning. The feces were collected in the evening. The feces were fermented by a rotator (8 rpm) for 14 days under 23°C. Algal growth potential (AGP) of this fecal medium was determined by using culture of *Nannochloropsis oculata*. The culture experiments of *N. oculata* were conducted in an incubator adjusted to 23°C, 7500 lux and 12L:12D. The AGP tests were repeated seven times in the Faculty of Agriculture, Kinki University, during January and March 1995. The maximum population density was used as AGP throughout the culture experiments. At the end of the experiments, water qualities such as NH₃-N, NO₂-N, NO₃-N and PO₄-P in the fecal medium were measured

RESULTS AND DISCUSSION

The results which showed relatively higher AGP throughout the experiments are presented in Figs. 1 and 2. In trial 2, the U-Y mix group showed a higher population density of 86.6 million cells/ml. The population densities in *Ulva*, yogurts and DP groups were 68.5, 48.3 and 42.2 million cells/ml, respectively. In trial 4, the *Ulva*-supplemented group showed the highest population densities through all the trials as 93.4 million cells/ml (Fig. 2). The densities in the U-Y mix, yogurts and DP groups in trial 4 were 88.6, 76.6 and 30.0 million cells/ml, respectively. The results obtained in each trial are summarized in Table 1 and Fig. 3. The highest population density was found in

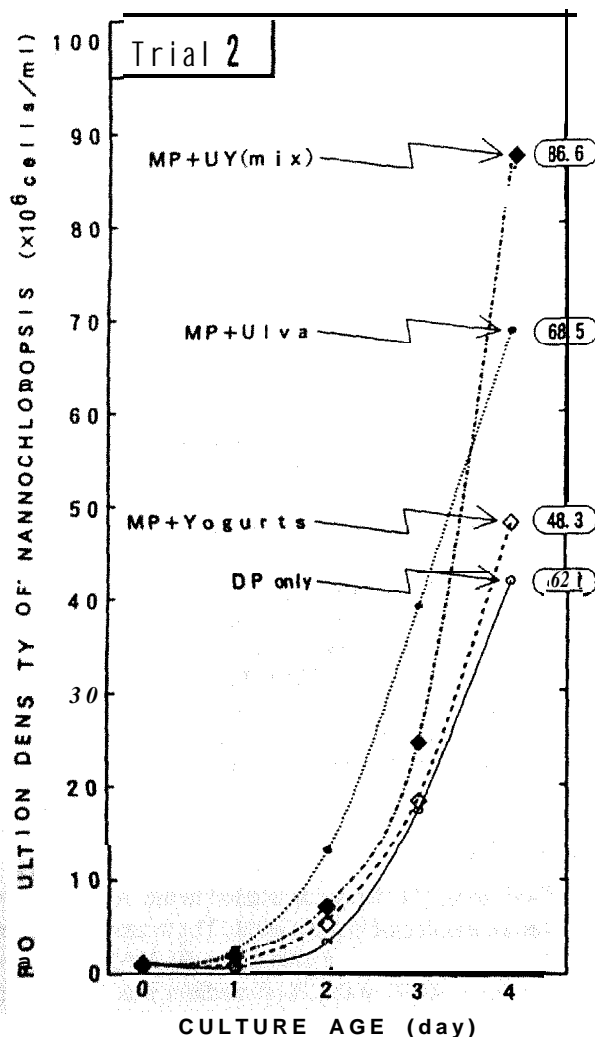


Fig. 1. Population growth of *Nannochloropsis oculata* cultured by the fecal medium obtained from red sea bream fed on probiotic supplements (2nd trial).

the U-Y mix group which showed 71.4 million cells/ml an average. The second highest density, 68.5 million cells/ml, was observed in the *Ulva* group. The third measurement was yogurts. The final fourth one was observed in the DP group. The DP group showed lower growth rates throughout the experiments.

Table 2 shows the results of water quality analysis observed at the end of the experiments. IN/IP ratios in the yogurts, *Ulva*, U-Y mix and DP groups were 9.9, 15.2, 72.5 and 190.5, respectively. The ratio in the DP group was remarkably high. It may be concluded that the higher IN/IP ratios caused lower algal growth rates.

Higher algal growth rates should reflect the fast material recyclings in the waters. When the fish were fed on *Ulva* and/or yogurts, each group showed higher algal growth rates. Therefore, those probiotic treatments are effective for fast material cyclings in the waters. This system could be termed an environmentally friendly culture

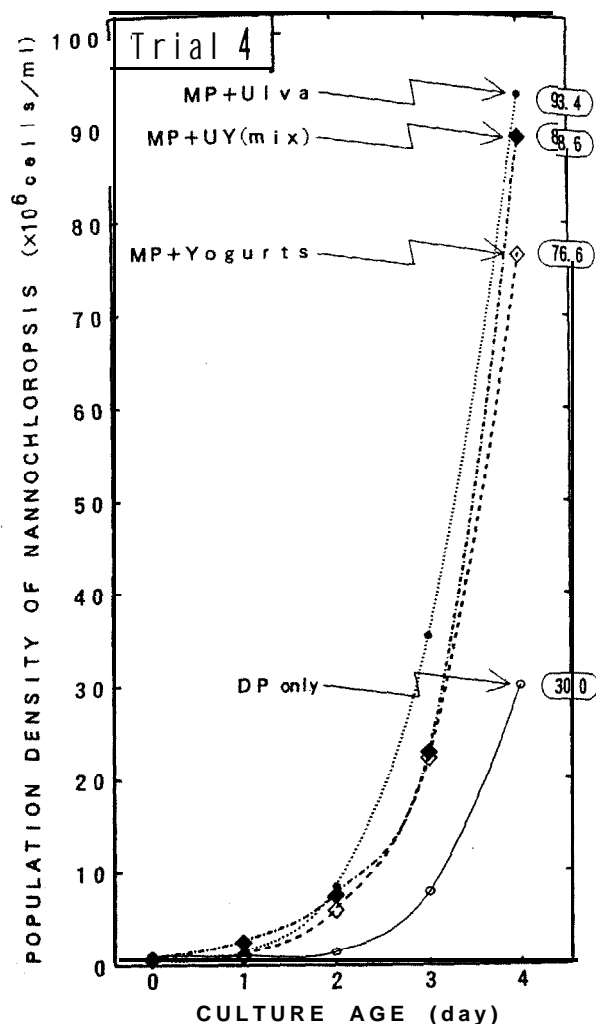


Fig. 2. Population growth of *N. oculata* cultured by the fecal medium obtained from red sea bream fed by probiotic supplements. (4th trial)

method. Recently, the sustainable aquaculture systems based on the homeostasis of ecosystems have been studied by several biologists (Ackefors 1990, Hirata 1989, Lee 1995). The problem is how to promote the energy flow in the sea farms. Nagahama and Hirata (1989), Xu and Hirata (1990), Hirata et al. (1994), Yamauchi et al. (1995), and Matsuda et al. (1996) have studied the feedback culture system by the polyculture of *Ulva* and red sea bream and/or Japanese flounder, *Paralichthys olivaceus*. According to their reports, when the fish were fed back on 2% of the sterile *Ulva* per diet, the growth rates increased 1.5%, with higher survival rates. Dissolved oxygen contents in the polyculture cage increased 9%. The carbon dioxide in the cage decreased 4%. The *Ulva* group in the present experiment reconfirms the results obtained in the reports mentioned above.

Probiotic Culture systems have been developed in the fields of animal husbandry (Fukushima and Nakano, 1995).

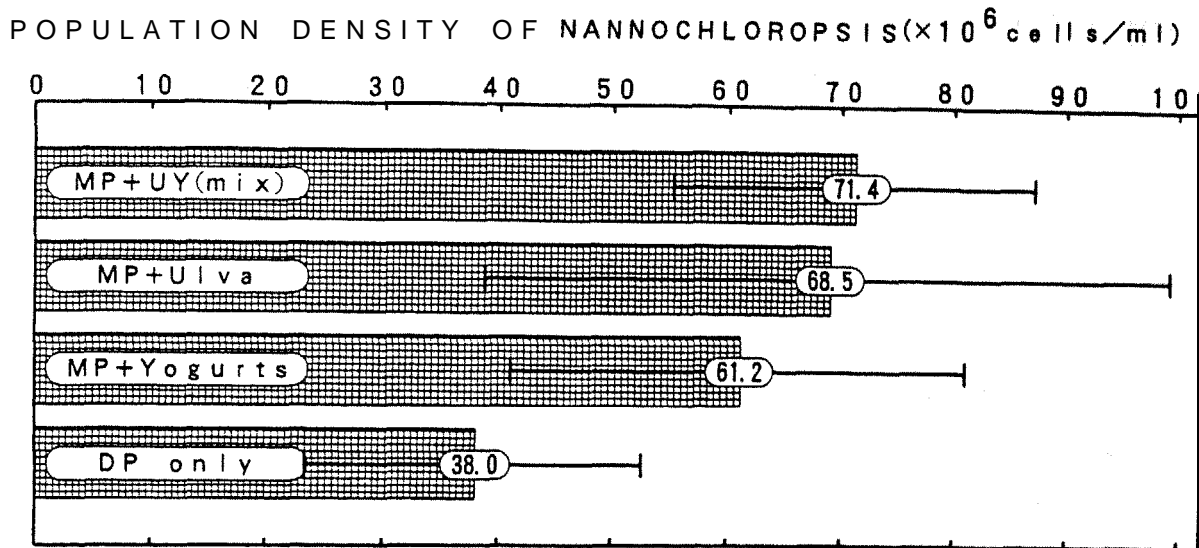


Fig. 5. Average of the first to seventh trial on the population growth of *N. oculata* cultured by the fecal medium obtained from red sea bream fed on probiotic supplements.

Table 1. Algal growth rates of *Nannochloropsis oculata* in each trial. (MP: Moist Pellets, DP: Dry Pellets, U: Ulva, Y: Yogurts)

	MP+Ulva	MP+Yogurts	MP+UY (mix)	DP only
	(x10 ⁶ cells/ ml)			
Trial 1	41.2	44.8	53.0	
Trial 2	68.5	48.3	86.6	42.2
Trial 3	66.0	63.0	79.3	48.5
Trial 4	93.4	76.6	88.6	30.0
Trial 5	42.2	45.6	46.2	25.4
Trial 6	33.6	44.9		17.7
Trial 7	134.8	105.3	14.7	64.0
Average	68.5	61.2	71.4	38.0
S D	33.1	21.1	16.2	15.5

Table 2. Inorganic nitrogen and phosphate contents in the fermented fecal medium. (IN: Inorganic Nitrogen, IP: Inorganic Phosphate)

	NHCN	NO ₂ -N	NO ₃ -N	PO ₄ -P	IN/IP
	(μg-at/l)				
Yogurts	5049	tri.	tli.	509	9. 9
Ulva	17909	tri.	tli.	1178	15. 2
UY (mix)	24735	tri.	tri.	341	72. 5
Control	48589	tri.	tri.	25.5	190. 5

In the case of aquaculture, however, the probiotic research field has just been initiated. The yogurt supplements in this experiment **could** possibly be applied to the probiotic techniques in aquaculture.

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Monitoring Systems Useful in Mass Production of Larvae for Japanese Fish Culture

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ABSTRACT

Recognizing a need for quantitative and labor-saving management in fish culture technology, we describe monitoring systems actually in use for continuous measurements in mass production in Japan, with special reference to two systems for water quality regulation: **first**, a basic system of independent and centralized measurement types for selected water quality parameters (temperature, hydrogen-ion concentration, and dissolved oxygen), and, second, a system for regulating the phytoplankton culture medium. Chemical and optical sensors contribute together to the functioning of these systems. Effective integration of these newer monitoring methods with empirical ones now in use should be the next step in automated monitoring.

INTRODUCTION

The mass production of fish fry has expanded in Japan to reach a level of more than one million fry a year in most of the fish culture facilities which rear such species as the red sea bream, *Pagrus major*, and the Japanese flounder, *Paralichthys olivaceus*. Nevertheless, such mass production has not been easy, because of some difficulties in controlling the process, even if the latest technology was used. This problem has indicated a need for a focus on establishing in these facilities monitoring systems for continuous measurement with quantitative and labor-saving capabilities.

We here describe the monitoring systems actually in use at related facilities in Japan, with special reference to two models for water quality regulation—one effective for basic parameters of water quality and the other for medium regulation in phytoplankton culture.

BASIC WATER QUALITY MONITORING SYSTEM

MONITORED SUBJECTS

Fish farming facilities now use continuous measurement of temperature, hydrogen-ion concentration (**pH**), and dissolved oxygen (DO) for water regulation in culture tanks, especially for brood stocks and larval breeding. In those continuous measurements, water temperature is always fundamental, and it has been measured for a longer time period than the other parameters. Recently, the other two **parameters**—**pH** and DO—have become popular items for continuous measurement. Earlier, Fujita et al. (1982) developed a continuous measuring system of water **quality**, including temperature, **pH**, and DO as well as **flow rate** in the fish fry tanks at the Kagoshima Prefectural Fish Farming Center, **Tarumizu**. They did not, however, em-

phasize the importance of other parameters, such as salinity and turbidity.

MODEL TYPES AND DESIGN

Up-to-date models of the continuous measurement system may be classified into the following-types 1 and 2—from the merit-rating viewpoint. The type 1 system consists of three parts (**Fig. 1**): sensor, monitor, and receptor or computer parts. The sensor **part** is composed of a set of sensors and their terminal devices. Every tank to be measured is furnished with the set. The terminal device converts sensor signals, and transmits them at 4-20 **mA** to the monitor. This part may receive sensor signals from a maximum of 16 terminals, display available measurements, convert analog signals to digital ones, and transmit these data to the receptor. This final part is a personal computer system **which** on the one hand displays on the cathode-ray tube necessary information, that is, measurement-related graphs, indicated alarms, and daily and monthly reports, and on the other hand stores available data. Alarm signals are automatically reported from the receptor to programmed addresses through an emergency warning device.

This type of system is rather simple to assemble and it provides continuous data at selected intervals. Another advantage of this type of system is its compensability. If any part of the sensor fails and does not transmit signals, other component parts may transmit an effective amount of signals. In fact, the most accepted system in Japan is this type.

If we consider the type 1 system independent in the **disposition** of sensors, the type 2 system (**Fig. 2**) provides a centralized disposition of sensors. The type 2 system consists also of three parts: sampling, sensor-monitor, and receptor. Sample water is conveyed for measurement from

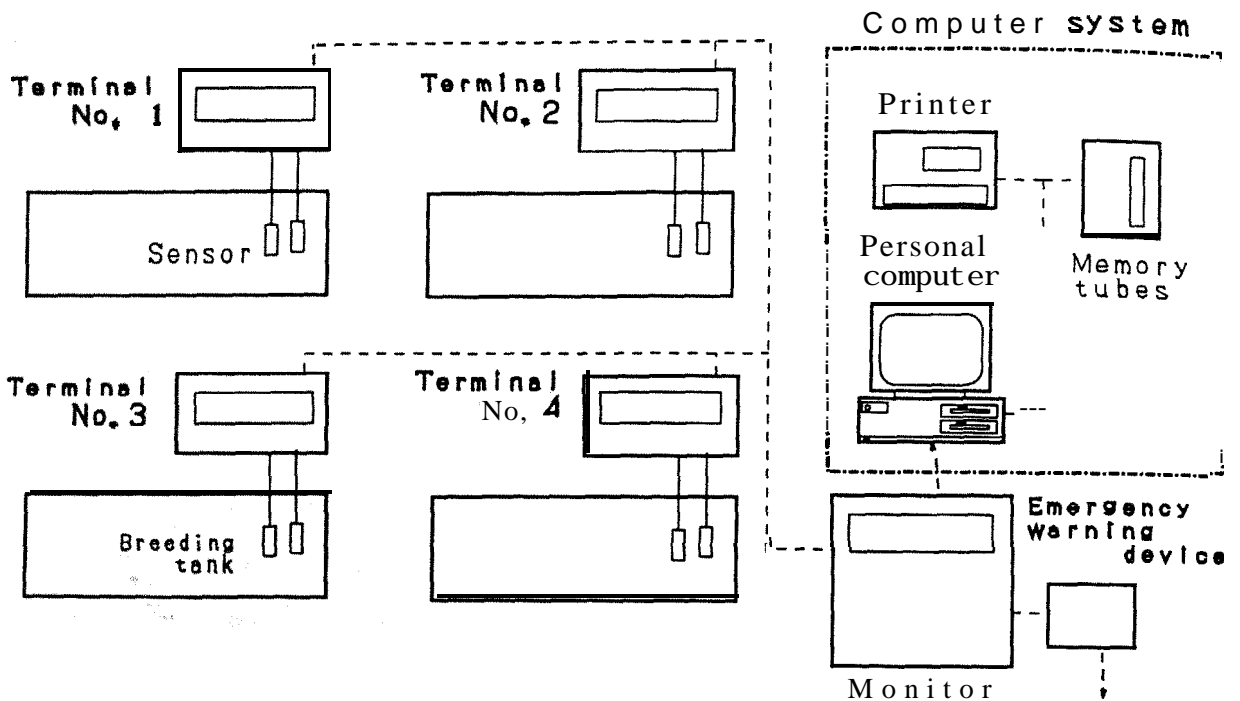


Fig. 1. Composition of the monitoring system model type 1.

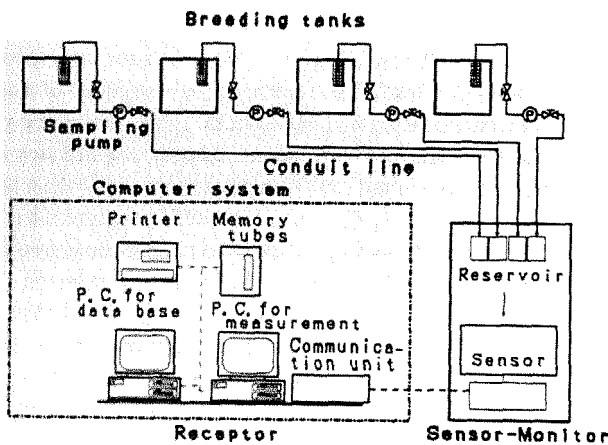


Fig. 2. Composition of the monitoring system model type 2.

a group of tanks up to the sensor-monitor by a pumping apparatus attached to each tank through a conduit line. The sensor-monitor is regulated to accept the sample water into a reservoir at intervals from the respective tanks for measurement. As soon as the reservoir is filled with water, the water flows down automatically into the succeeding tank with a set of sensors for common use. The set is composed of sensors which measure temperature, pH and DO, as well as some optical parameters of water quality. This optical sensor unit will be illustrated in detail later. Additional functions of this part of the system may be (1) automatic cleaning of the tank containing the sensors, and (2) an alarm setting for regulation of given parameters within the programmed range. Available data from this part are converted

from analog to digital signals, and transmitted to the receptor through a communication apparatus. The final part, or a computer system, stores and controls continuous measurements and other necessary data as a functional data base for breeding management.

The type 2 system offers some advantages; first, it economizes on sensors which may be very expensive, and reduces professional maintenance, such as regulation of delicate sensor units; second, it easily gives higher precision to the data, because each parameter is measured in this system by a single sensor unit, and most of the measurement errors among different units may be eliminated.

Kanamaki and Shirojo (1994) tried to develop a type 2 model (Fig. 3), applying an up-to-date sensor unit of optics, under the following circumstances: during early stages of fish breeding, phytoplankton species of *Nannochloropsis* are usually added to the rearing water to feed therotifers as well as to improve water quality. Concerning the feeding of the animal, inspectors determine when and how much phytoplankton should be added. Too much may cause a persistence of prey organisms in the water; this may result in a sudden deterioration of water quality by rapid mortality of the prey organisms. To regulate the addition of phytoplankton, concentrations may be controlled on the basis of numerical data provided as relative fluorescence intensity by a sensor for chlorophyll a with an excitation wavelength of 436 nm and an emission wavelength of 685 nm. This sensor may be used to measure detritus concentrations in combination with a beam transmittance sensor for near infrared rays.

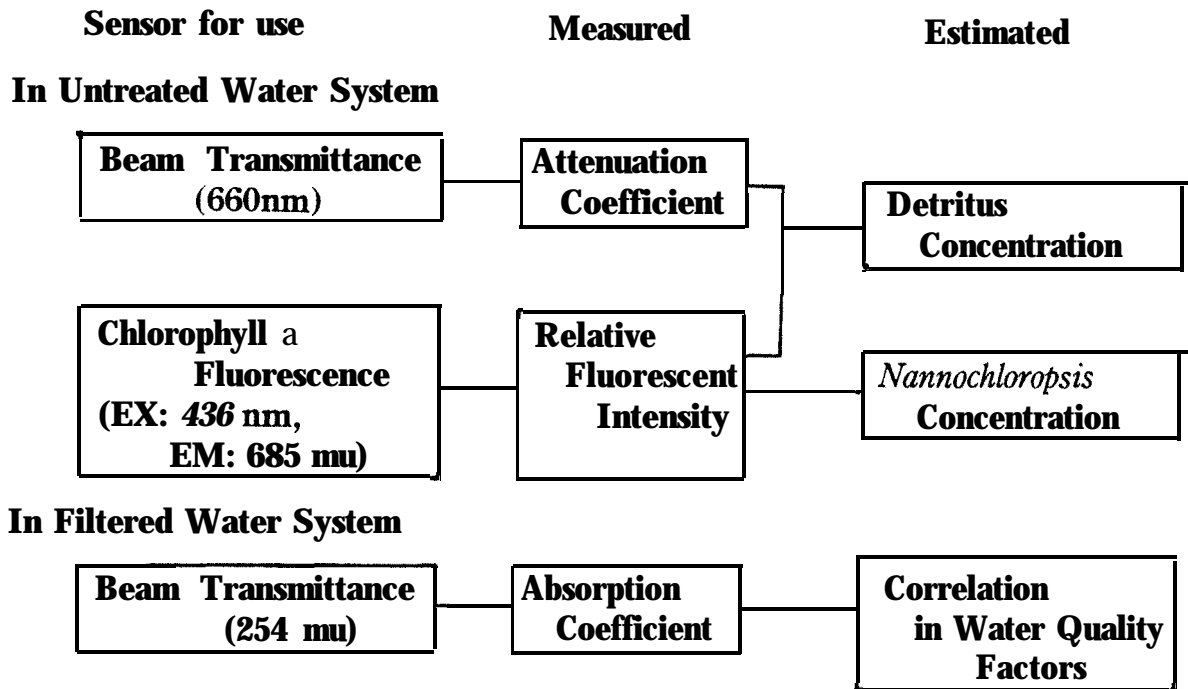


Fig. 3. Flow diagram of water quality monitoring by optical measurement. Columns: left, sensor series; middle, measured properties; right, estimated properties. EX: excitation wavelength; EM: emission wavelength (Kanamaki and Shirojo 1994).

During the period of larval breeding in standing water, the feeding of the rotifers eventually reduces water quality in the breeding tank. Preventive measures for this trend are to exchange water appropriately just after the period when larvae grow enough to adapt to a stronger flow during water exchange, and just before a lethal deterioration of water quality. This larval breeding management technique has previously depended upon the empirical estimation of the technicians in charge.

As for the water exchange in larval breeding, numerical management is useful for this maintenance, depending upon continuous measurement of nitrogen in the form of ammonium ion ($\text{NH}_4\text{-N}$), and chemical oxygen demand (COD) in a dissolutive condition. Dissolved matter may be detected through the absorption coefficient of ultraviolet rays in filtered water. This coefficient is measured by a beam transmittance sensor. Water pollution in the rearing water then may be indicated by the absorption coefficient of ultraviolet rays in relation to the concentration of the dissolved matter.

In the field of marine science, optical sensors have been developed especially for oceanographic observation and for biotechnological management in fish culture, and have become available in small-sized and moderate-priced models. Similar models could be easily applied to water quality monitoring in aquaculture operations. The following are important current models: (i) a fluorescence intensity sensor (Fig. 4) designed by the Fuyo Ocean Development and Engineering Co., Ltd., Tokyo, Japan, especially for continuous measurement in a high density; its size is

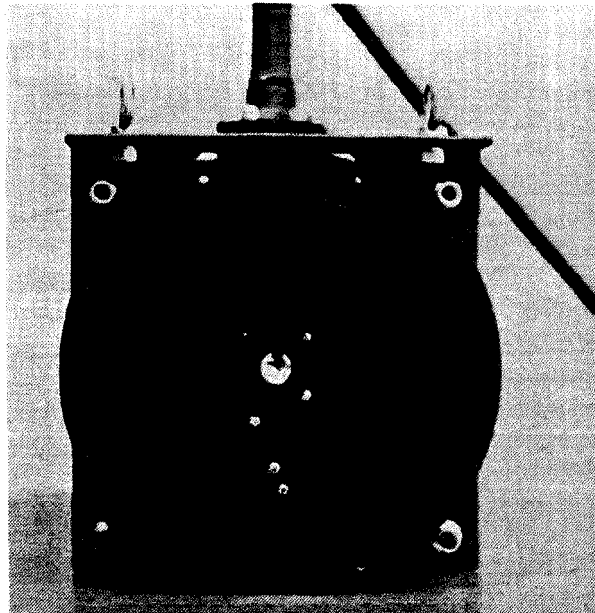


Fig. 4. A fluorescence intensity sensor unit for continuous measurement. A wiper is provided to keep clean its light-slanting window at the center of the body.

30 cm in diameter and 45 cm in length; (ii) a smaller sensor (Fig. 5) with the same function as above for oceanographic observation, designed by the ALEC Electronics Co., Ltd., Kobe, Japan; (iii) a sensor (Fig. 6) of the same function and purpose as above, designed by the Western Environment Technology Laboratories, Inc., Philomath,

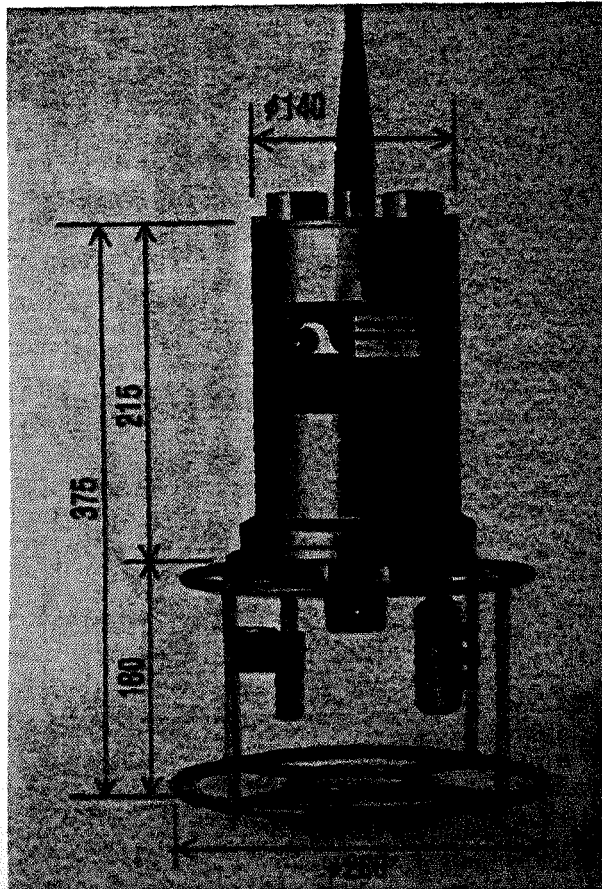


Fig. 5. A fluorescence intensity measuring instrument for oceanographic observation. Sensor assemblage: at the foot part (from left to right), sensor for salinity, fluorescence intensity, and turbidity; inside the body, sensors for water temperature and depth (catalog of the LEC Electronic Co., Ltd., Kobe, Japan).

OR, USA; (iv) a turbidometer (Fig. 7) with scattering and transmittance light measurement functions, designed by the Automatic System Research Co., Ltd., Tokyo, Japan, to monitor the cell numbers of cultured microorganisms (Yamane 1993).

MATHEMATICAL BASIS FOR MONITORING ALGAL DENSITIES

To estimate the concentration of *Nannochloropsis* species of detritus, the following series of equations is applicable.

The relationship between *Nannochloropsis* density (N) and fluorescence intensity (F) is given in equation (1), and total attenuation coefficient in equation (2) as a function of N and detritus concentration (D).

$$F = \alpha N \quad (1)$$

where α is a proportional coefficient.

$$(c - c_w)\lambda = \beta_\lambda N + \gamma_\lambda D \quad (2)$$

where c is total attenuation coefficient of culture water;

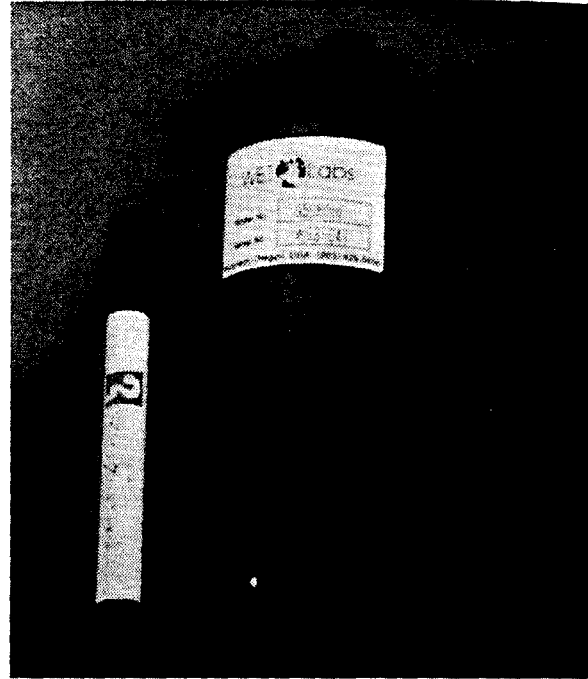


Fig. 6. A smaller fluorescence intensity probe (right) for oceanographic observation (catalog of CT and C Co., Ltd., Tokyo, pan).



A turbidometer unit for monitoring the cell number of microorganisms. Parts assemblage (from left to right): controller and a set of probes (high-temperature-resisting-type and standard one) (catalog of the Automatic System Research Co., Ltd., Tokyo, pan).

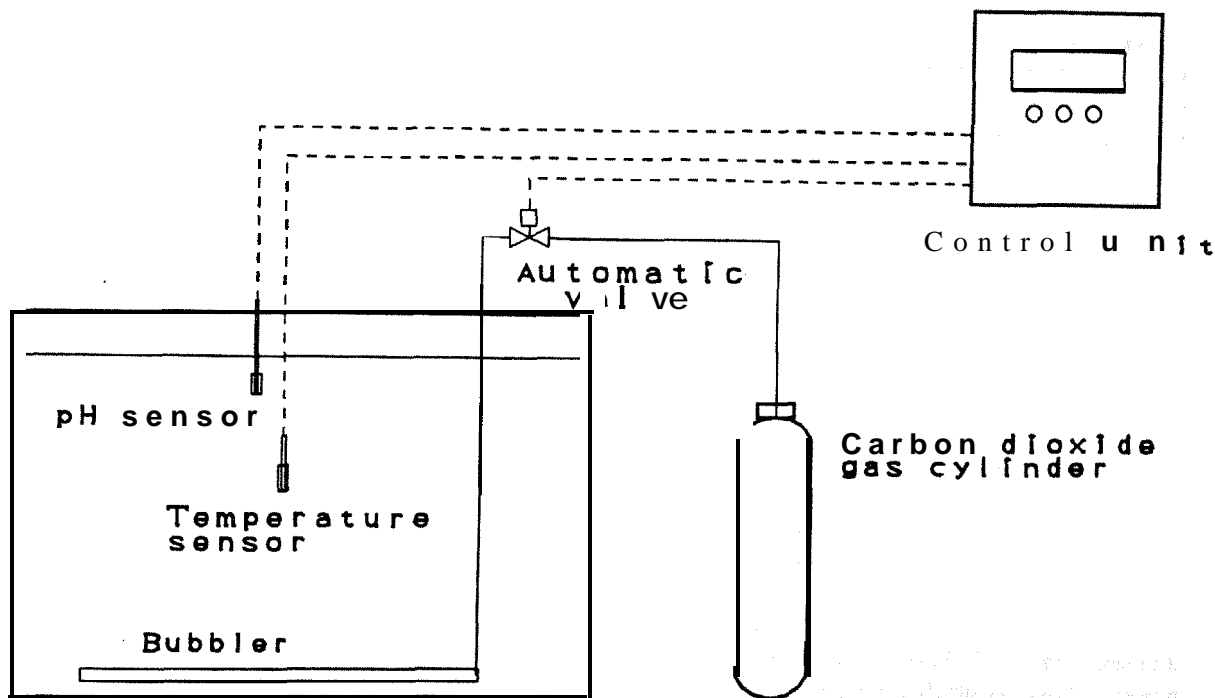


Fig. 8. A forced gas feed system composition for high density culture of phytoplankton.

c_w , total attenuation coefficient of pure water; λ , wavelength; β, γ , proportional coefficients.

In these equations, the terms for dissolved matter and rotifers are negligible. In these interrelationships, the equation (1) gives the fluctuation of the plankton density. This is derived by measuring fluorescence intensity, F , because α may be previously given on a working curve of F and N . The detritus concentration, D , is given from the interrelationships shown in equations (1) and (2); the elimination of N in these expressions gives equation (3), or an interrelationship between F and the left side term $(c - c_w)_\lambda$.

$$(c - c_w)_\lambda = \frac{\beta}{\alpha} F + \gamma D \quad (3)$$

These equations may give total attenuation coefficient, γD , continuously in relation to D , because either c or F is a continuous measurement.

MEDIUM REGULATING SYSTEM FOR PHYTOPLANKTON CULTURE METHODOLOGY

The second monitor system introduced here is a management system for phytoplankton culture through regulation of the culture medium. This culture is well known as a basic requirement for feeding of fish larvae. Recent efforts have been aimed at establishing a high density and stable culture of nutritive phytoplankton by adding carbon dioxide gas into the culture medium, in order to control photosynthesis, Hamasaki and Maruyama (1991) studied the effect of carbon dioxide gas added to

Nannochloropsis culture, and they suggested that it is useful to add the gas into the culture medium especially during a high water temperature period. An adequate solution has not been found, however, for the problem of how to obtain better effects from the gas supplement and how to regulate such a treatment appropriately, although feasibility studies have been started already on fish seed production by highly motivated researchers (Osawa and Nagano 1994).

As for our experimental application (Fig. 8), the gas supplement is regulated by an on-off control in relation to the pH level in the culture tank containing sensors for temperature and pH. As another aspect of our experiment, we used an optical device, or beam transmittance sensor of near infrared rays, to measure continuously the density of cultured phytoplankton. Available data are derived from the transmittance of a parallel ray pencil of 690 nm in the culture water. The transmittance can be converted for suitable analysis to total attenuation coefficient as given in equation (4).

$$(c - c_w)_\lambda = \frac{1}{L} \ln(K_\lambda \frac{I_a}{I_w}) \quad (4)$$

where L is the path length; K_λ , a correction factor; I_w , value in the water; I_a , value in the air.

In the case of the 690 nm wavelength light, optical absorption by dissolved matter is so little as to be negligible in the culture medium. The total attenuation coefficient concerned with the phytoplankton, c'_{690} , may be given by equation (5).

$$c'_{690} = c_{690} - c_{b,690} \quad (5)$$

where the background estimate, $c_{b,690}$, is deducted. The total attenuation coefficient, c'_{690} , may fluctuate in proportion to the density and projective area of planktonic cells as shown in equation (6),

$$C'_{690} = \sum \Omega_{690} n A \quad (6)$$

where Ω_{690} is efficiency factor; n , density in number of *Nannochloropsis* species per unit volume; A , projective area of the cells.

Consequently, at a light intensity period when cells do not increase in number, fluctuation of the phytoplankton growth apparently depends on the projective area, in close relation to the cell growth in diameter. On the contrary, considering the behavior of daily periodical measurements, the projective area is almost inactive at every daily measurement. This means that the coefficient is affected exclusively by the cell numbers in this case. Based on equation (7), the specific growth rate, μ , is optically estimated with the aid of c'_{690} , or continuous measurements of the total attenuation coefficient in relation to the phytoplankton growth,

$$\frac{1}{c'_{690}} \frac{dc'_{690}}{dt} = \mu F \quad (7)$$

where $F = 1$ in the exponential growth phase; $F = \exp\{-\delta(t - t_d)\}$ in the decreasing growth phase; μ , specific growth rate in the exponential growth phase; δ , decreasing growth factor; t_d , days elapsed before the decreasing growth phase.

As referred to already, the variable c'_{690} is proportional to the phytoplankton cell numbers. Therefore, equation (8) can be formed for the growth curve in the exponential growth phase, and in this case the rate is regarded as constant:

$$\frac{1}{c'_{690}} \frac{dc'_{690}}{dt} = \mu \quad (8)$$

In the decreasing growth phase, equation (9) indicates that the rate decreases exponentially with the passage of time.

$$\frac{1}{c'_{690}} \frac{dc'_{690}}{dt} = \mu \exp\{-\delta(t - t_d)\} \quad (9)$$

The change seen in the phytoplankton cell numbers may be deduced by applying this equation to the growth process of the organisms.

AN APPLICATION

In some of our experiments with synchronized culture for *Nannochloropsis*, fluctuations in specific growth rates seem to reveal an interesting fact (Fig. 9). In those cases, the trend is apparently divided into two phases: a stable or constant phase and a straightly decreasing one. The former phase corresponds to the change in the exponential growth phase referred to in the equation mentioned above, and

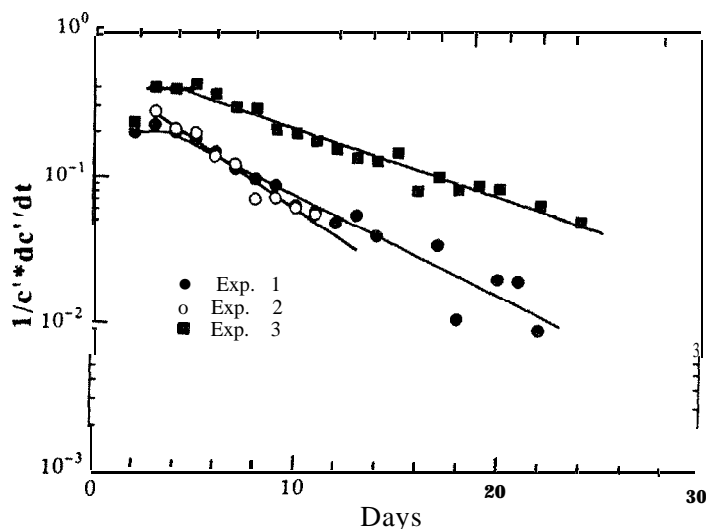


Fig. 9. Relationships of specific growth rate (ordinate) with the culture period (abscissaa, days).

the latter one corresponds to the change in the decreasing growth phase in the same case. In other words, the equation, especially in the latter phase, approximates effectively the phenomena concerned.

Based on the regression lines obtained from experiment No.3 (Fig. 9), the cell density in the stable condition may be estimated to reach the level of 1300×10^4 cells/ml.

Our information indicates that the continuous measurement of total attenuation coefficient will give us, with the succeeding analysis of the growth curve, the attractive possibility of monitoring the duration of the stationary period and the cell number of the phytoplankton of *Nannochloropsis* species.

SUMMARY AND CONCLUSION

1. The present condition of continuous measuring systems for fish larval production in Japan is briefly reviewed from the viewpoint of their functions.
2. Current models of the monitoring system are effective for important parameters of water quality as well as for regulating the medium in phytoplankton culture.
3. These models are described in terms of their composition and peculiarities, with necessary mathematical background for monitoring sensors.
4. Advanced systems for the quantitative control of fish larval production will depend on an effective integration of numerical data available from the systems in question with those provided by empirical technologies for breeding management, through further development of electronic monitors.

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Reducing the Environmental Impact of High Density Fish Production: An Integrated Approach to Solids Treatment for Recirculating Aquaculture Systems Using Expandable Granular Biofilters

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ABSTRACT

Over the past decade, regulatory agencies have begun to view the environmental impact of wastes discharged from high density flow-through fish production systems with increasing concern. At the same time, recirculating aquaculture systems have gained wider acceptance because of their ability to reduce waste discharges, improve quality control and reduce costs. The crucial processes that must be addressed in treating recirculating water are solids capture, biofiltration, aeration, degasification and ion balance. Designs that integrate two or more of these processes provide the greatest potential for cost reduction. The technology that is the focus of this paper is an expandable granular biofilter (EGB), which integrates solids capture and biofiltration in a single unit process. Backwash frequency is a major operational parameter of EGBs, influencing the volume of sludge produced and the nitrification rate. Computer and mass balance models are used to describe the relationship between solids residence time, sludge production and nitrification rates. The models show that infrequent backflushing decreases water loss and sludge production, although nitrification rates decline for extended solids residence times. Declining nitrification rates reflect decay of the accreting solids mass-which creates an internal ammonia and BOD load, decreasing the oxygen available to the nitrifiers because of heterotrophic competition for oxygen and impeded mass transfer as the bed becomes occluded. Nitrification appears to be optimized with solids residence time in the range of 2-3 days, for filters utilized as the primary solids capture device. The focus of this paper is: (1) primary in-filter solids stabilization; (2) the effect of in-filter solids stabilization on nitrification; and (3) post-discharge, pre-disposal digestion of aquacultural solids.

INTRODUCTION

As waste discharges from flow-through systems have become the subject of increasing scrutiny by government regulatory agencies, recirculating systems are increasingly seen as a potentially effective means of minimizing the impact of large-scale aquaculture on water and environmental resources. High density recirculating aquaculture systems (RASs) allow for systematic optimization of costs and the environmental variables that determine the quality of the targeted aquatic species. Fish culturists in the

United States are adopting RASs to enhance the production of highly valued products including tropical fish, ornamental goldfish and soft crabs. Researchers have been working toward integrating the functions and improving the efficiency of recirculating system components. The goal for recirculating systems research is extending the economic viability of RASs to commodity-priced products.

The five processes required to recondition water in a recirculating system are solids capture, biofiltration, aeration, degasification and ion balance. Solids capture is the

removal of feces, uneaten food and suspended bacteria, and it can be performed by settling tanks, **microscreens** or **granular filters**. **Biofiltration** is the conversion of **dissolved organics** and toxic nitrogenous compounds to **bacterial biomass**. Several common **biofilters** are rotating **biological contactors (RBCs)**, trickling filters, fluidized beds and granular beds. Aeration is often provided by blowers and air stones, and it must be sufficient to meet the respiration demands of the culture species and the bacteria in the **biofilter**. Degasification is the removal of carbon dioxide that results from respiration in the system. Degasification can be achieved concomitantly with aeration by **sparging** a sufficient volume through air stones, or it can be **performed** separately in a packed column. Ion balance is **necessary** to **correct** potentially harmful chemical imbalances in systems where the water exchange rate is very low. Ion balance may be achieved by increasing the water exchange rate, **addition** of chemicals or **denitrification** for nitrate removal. Each of **these crucial** processes must be addressed to provide minimally acceptable water quality for the **culture** species.

System efficiency can be increased by the addition of **optional** processes: foam fractionation, ozonation, W **disinfecting** or **denitrification**. Foam fractionation removes **organics** that are not easily oxidized by bacteria, which helps control fine solids ($<10\ \mu$). A foam **fractionator** is simply a tube **containing** air stones, where fine particles form foam **at** the air-water interface for removal at the surface. **Ozone** is an effective oxidant used to remove **refractory** organic **material**, which contributes to color **problems** and **bacteria**. **Ultraviolet (UV)** light can be used to **control** many forms of bacteria algae and some viruses, **reducing** disease outbreaks. Denitrification addresses ion **imbalance** created by the **nitrification** process, removing excess nitrate and replenishing alkalinity. These processes, although usually considered optional, may become **mandatory** if stocking density is very high, the water reuse period is extended or endemic disease becomes a **problem**.

EXPANDABLE GRANULAR BIOFILTERS

Solids capture and biofiltration are often performed as **sequential unit operations** in **RASs**. For example, a **settling basin** followed by a rotating biological **contactor** is a **widely-used configuration** (Libey 1991, Van Gorder 1991). An expandable **granular biofilter (EGB)** performs **both processes** in a single operation. Early EGB **configurations**, such as the **upflow sand filter** (Burden 1988, Malone and Burden 1988), were limited in total **ammonia nitrogen (TAN)** conversion and solids capture by the **fluidization characteristics** of the sand. These limitations were **overcome** in **EGBs** through the use of low-density floating **plastic** beads. Bead filters exhibit superior oxygen transport

and are more easily cleaned than sand filters. Filtration of total suspended solids (TSS) is accomplished by **settling**, **straining** and **interception** within the granular bead **matrix**, and the bead bed operates simultaneously as a fixed film bioreactor. Periodic washing removes excess **heterotrophic** bacteria and highly organic solids, which **accumulate** in the interstitial spaces between the beads. Backwashing mitigates solids **ammonification** and **occlusion** of the filter bed, so it is the primary means of **optimizing filter performance**.

The benefit of this filtration approach stems from the extremely low water loss associated with solids removal, and the large specific surface area ($1100\ \text{m}^2/\text{m}^3$) provided for the growth of bacteria. Low density polyethylene beads (3-5 mm in diameter) are employed as a filter media in an **upflow** pressurized configuration. The beads are less dense than water, float above the injection line and are retained in the filter by an overlying stainless steel screen. A **propeller**, embedded in the filter media, is activated for **periodic** cleaning. The filtration bed is underlain by a **cone-shaped** settling chamber, and as shown in Fig. 1, the filter has four operational modes.

During a typical filter operation (Step 1), **nitrifiers**—which convert toxic ammonia and nitrite to stable **nitrate**—and heterotrophic bacteria—which remove biochemical oxygen demand (**BOD**)—**become** attached to the filter media. Heterotrophic bacteria, which grow more rapidly, soon fill the pore spaces between the beads. As solids and bacterial biomass accumulate, solids **ammonification** increases and the transfer of oxygen and nutrients to the **bacteria** in the filter is impeded. Triggered by a timer, **pressure** sensor or computerized control unit, the **propeller-driven** backwash sequence is implemented, **homogenizing** the bed into the underlying settling chamber (Step 2). When the propellers are turned off, the beads float upward reforming the **filtration** bed, while the accumulated solids and the **bulk** of the heterotrophic bacteria become **concentrated** in the bottom of the settling chamber (Step 3). While a large **part** of the solids accumulating in the settling cone consist of bacterial biomass that are grown in the **inter-vening** filtration cycle, a portion of the nitrifying and **heterotrophic** bacteria remain attached to the beads. After **backwashing**, the solids are allowed to compress, forming a concentrated sludge that can be removed with only **minimal** water loss (Step 4). Since water loss is negligible, the solids can be harvested frequently and little of the **particulate BOD** from solids excretion is expressed in the **system**. Thus, the bead filter is capable of mitigating extremely **high wasteloadings**.

Bead filters compare quite favorably to trickling filters and **RBCs**, which are clearly effective biofiltration units (Table 1). However, trickling filters and **RBCs** must **maintain** high porosity to avoid **biofouling**, which limits their specific surface and thereby their volumetric conversion

Table 1. A comparison of TAN conversion rates for common biofilter types.

Filter type	Areal TAN conversion (g/m ² -day)	Specific surface area m ² /m ³	Volumetric TAN conversion g/m ³ -day)	Reference
RBC	0.280	150	41	Mississippi Power and Light demonstration facility, Greenville, MI (Malone <i>et al.</i> 1993)
Upflow sand	0.064	2350	152	Commercial softcrayfish facility (Burden 1988)
Hydraulically washed EGB	0.23 1	1230	286	Experimental scale cattish system (Wimberly 1990)
Mechanically washed EGB	0.291	1050	308	Mississippi Power and Light demonstration facility, Greenville, MI (Malone <i>et al.</i> 1993)
Fluidized bed	0.284	2350	633	Experimental scale chemically fed (Thomasson 199 1)

capacity. On the other hand, fluidized beds display superior volumetric nitrification rates, but they must be used in conjunction with a solids capture device. Use of a bead filter for nitrification, in lieu of a **fluidized** bed, is predicated on the assumption that integrated treatment is more cost effective. That is, a bead filter sized for nitrification will be less costly than a properly sized solids capture device and a fluidized bed.

SLUDGE PRODUCTION

All the sludge generated from a recirculating **aquacultural** system can be equated to the feed. Assuming a typical feed conversion ratio of 1-2 kg feed/kg fish, and neglecting the impact of uneaten food, 80% of the feed (on a dry mass basis) put into an aquacultural system will eventually be wasted as fish excretion products (Hopkins and Mancini 1989). Sludge volume is a major factor in designing a waste treatment system for effluents. Sludge volume generated from a recirculating system is controlled by the amount of solids produced (measured as kg of dry weight solids) and the degree to which the solids are concentrated in the effluent stream. Total sludge production from a recirculating system can be estimated by considering direct fish excretion, solids breakdown and biofloc production from soluble BOD excretion. The concentration is controlled by the solids removal technique employed to capture solids from the recycled stream. Solids production can be quantified through a mass balance that considers the major solids fluxes:

$$d(M_s)/dt = F \cdot M_o (E_s + E_b \cdot Y_H) - k_s \cdot M_s \cdot S_v - H_s \cdot M_s \quad (1)$$

Where:

M_s is the mass of solids in the filter bed (mass);
 M_o is the mass of cultured species (mass);
 E_s is the solids excretion ratio (mass **TSS**/mass feed);
 E_b is the dissolved BOD5 **excretion** ratio (mass **BOD5**/mass feed);
 Y_H is the heterotrophic yield (mass **VSS**/mass **BOD**);
 k_s is the solids decay rate (d-l);
 S_v is the volatile solids fraction (unitless); and
 $H_s = h_f f_b$ (2)

Where: H_s is the solids harvest rate (d-l);
 h_f is the solids harvest fraction (unitless); and
 f_b is the backwash frequency (d-l).

The direct solids excretion ratio (E_s) has been observed as 0.40 (Speece 1973) to 0.52 kg/kg feed (Liao and Mayo 1974) for trout and 0.43 kg/kg feed for **catfish** (Wimberly 1990). Other reported TSS excretion rates for catfish ranged from 0.18 to 0.69 kg/kg feed (Page and Andrews 1974, Ruane *et al.* 1977). Solids excretion rates clearly vary with species, temperature and feeding rates. However, values of E_s in the range of 0.3 to 0.5 are common.

The soluble five-day biochemical oxygen demand (BOD₅) excretion rate can also be expressed as a fraction of the feeding rate. Based upon a study of channel catfish, Murphy and Lipper (1970) reported the soluble BOD₅ as 58% of the total BOD, excreted, whereas, BOD₅ in particulate matter was 42%. Wimberly (1990) found that the soluble BOD₅ excretion ratio was 0.05 kg BOD₅/kg feed, or about 23% of the total BOD, excreted.

The first-order solids decay rate (k_s) at 20°C varies with solids residence time (SRT); k reportedly varies from 0.124 SRT^{-0.594} (Z. Ning, Louisiana State University, Baton

Rouge, pers. commun. 1995) to $0.278 \text{ SRT}^{-0.518}$ (L. Wang, Louisiana State University, Baton Rouge, pers. commun. 1995). The volatile suspended solids (VSS) fraction of catfish excretions has been reported as about 0.9 by Wimberly (1990) and as about 0.7 for Kemp's ridley sea turtles (Malone et al. 1990), red swamp crawfish (K.M. Cange, Louisiana State University, Baton Rouge, pers. comm. 1987) and blue crabs (Burden 1988).

The solid production from biofiltration depends on the growth of bacterial biomass during the breakdown of dissolved organics (BOD_5) and nitrification. Considering ammonia nitrogen excretion rates of 1.8 to 4.6% of the feeding rate (Page and Andrews 1974, Ruane et al. 1977, Wimberly 1990) and the stoichiometry of nitrification cited by Wheaton (1977), biomass production due to nitrification is negligible at 0.3% to 0.9% of the feeding rate. The biomass production due to dissolved BOD, consumption, on the other hand, is more significant. For example, if the soluble BOD, excretion is $0.05 \text{ kg BOD}_5/\text{kg feed}$, as reported by Wimberly (1990), biofloc production will be about 6% of the feed rate.

H_s is related to the solids residence time (SRT) by:

$$\text{SRT} = 1/H_s \quad (3)$$

The solids harvest rate for a given bead filter can be determined by washing the filter repeatedly and estimating the harvest fraction, and SRT can then be estimated for a variety of backwashing sequences. With a backwash frequency of once every two days, a filter with a harvest fraction of 0.5 will have a H_s of 0.25 and an SRT of 4 days.

The sludge production constant S_p (kg/day) from the system is defined as:

$$S_p = H_s * M_s \quad (4)$$

The concentration of the sludge stream (S_c , kg/m^3) is determined by the efficiency of the sludge separation process and the amount of flushing or washdown water (Q_s , m^3/day) required for the sludge removal.

$$S_c = S_p/Q_s \quad (5)$$

Calibration of a computer model, using Equations 1 through 5, resulted in $k_s = 0.665 \text{ d}^{-1}$ for an SRT of 3.5 days. Because aquaculture sludge is partially digested in the filter, k_s values for sludge with a high SRT are usually lower than those observed for municipal waste ($0.48 \text{ SRT}^{-0.415}$, Rich 1982).

Equations 1 through 5 can be used to estimate sludge production from a proposed recirculating configuration. Table 2 illustrates that the mass of total Kjeldahl nitrogen and the volume of sludge generated by fish is comparable to that of other commercially raised animals.

DISCUSSION

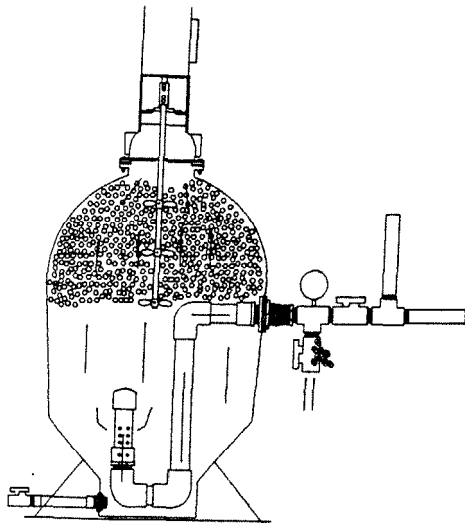
Direct discharge of untreated aquacultural solids to receiving streams, e.g., in flow-through systems, can cause

a variety of problems including oxygen depletion, nutrient enrichment, loss of water clarity and destruction of benthic communities by the formation of sludge deposits. However, in recirculating systems, water discharge rates are negligible, e.g., 5-10%/day, and effluent streams can be clarified, separating and concentrating settleable solids prior to discharge. Although the sludge itself must be disposed of through land application or landfilling, the material has been partially oxidized by biofilter bacteria. The two issues most important to successful aquacultural solids management are (1) managing primary in-filter solids stabilization to minimize its impact on other critical system functions and (2) selecting the most appropriate post-discharge treatment option.

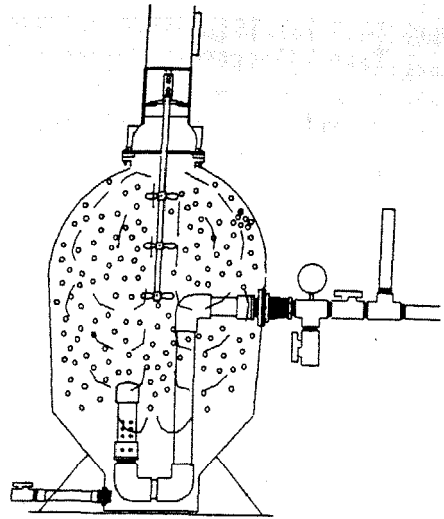
As can be seen from Equation 4, the mass of sludge produced from a recirculating system (S_p) is the product of the solids content of the system (M_s) and the sludge harvest rate (H_s). However, H_s and SRT are inversely related through Equation 3. Decreasing H_s tends to decrease sludge production as the amount of solids decay increases with SRT, but solids ammonification and mass-transfer constraints also increase with SRT, causing apparent nitrification to decline. Chen et al. (1993) applied Equations 1 through 3 to a model of a hypothetical finfish system of a 1000 kg capacity. Utilizing constant values for $E_s=0.4$, $E_B=0.05$, $Y_H=0.4$ and $k_s=0.36$, a reduction of about 50% in discharged sludge mass was achievable by manipulating the bead filter backwash frequency. However, the corresponding increase in sludge mass held in the system ultimately increases aeration and degasification burdens on the recirculating system and causes the apparent nitrification rate to decline. This phenomenon constrains efforts to manipulate SRT for purposes of sludge volume reduction. Naturally, the SRT-nitrification relationship will define the maximum sludge reduction and the backwash frequency should be manipulated to optimize nitrification.

Sludge management policies should focus on increasing S_c (Equation 5), since the concentration of solids varies dramatically with the type and management of the solids control device employed in the recirculating loop (Table 3). Additionally, the sizing criteria and cost of the stabilization and disposal options depend upon the volume of sludge produced, if the solids capture device in the production system is not capable of concentrating the solids, then an external clarifier should be used to achieve the desired sludge density. Consideration should be given to partitioning sludge stabilization between internal and post-discharge treatment processes. Integrated design allows for overall minimization of treatment costs, reduction of the potential for adverse environmental impact and enhanced RASE efficiency.

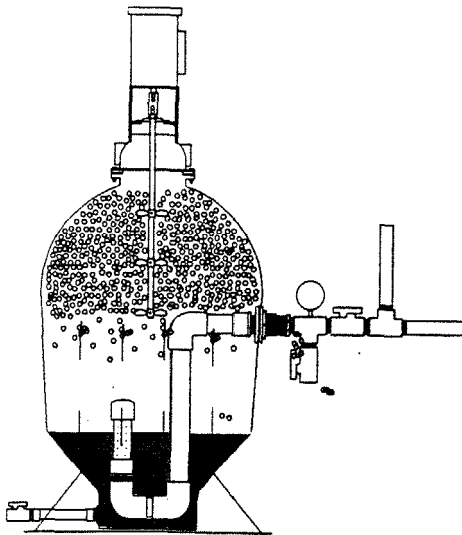
Bead filters are designed with internal settling cones, to facilitate single stage sludge concentration (Fig. 1). Additionally, the sludge retention time can be controlled



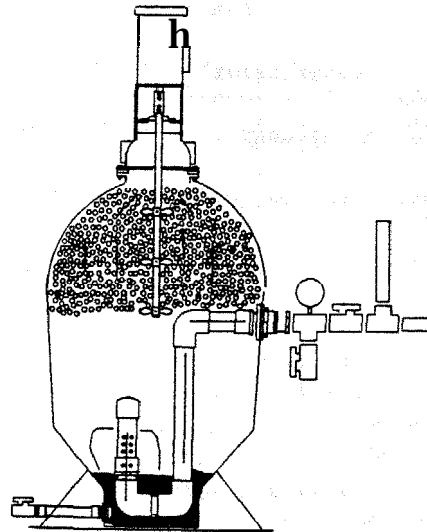
Step 1: Filtration



Step 2: Backwashing



Step 3: Settling



Step 4: Solids Removal

Fig. 1. The four operational modes of an expandable granular biofilter (EGB).

Table 2. Comparison of waste generation rates of commercial animals (kg/d).

Animal	BOD	TSS	TKNSludge	vol.	Reference
Fish	1.13	3.9-6.3	0.2-2.32	65-630	Chen <i>et al.</i> (1993)
Beef cattle	1.6	9.5	0.32	30	Middlebrooks <i>et al.</i> (1982), Overcash <i>et al.</i> 1983)
Dairy cows	1.4	7.9	0.51	51	“
Poultry	3.4	14	0.74	37	“
Swine	3.1	8.9	0.51	76	“

Table 3. A comparison of sludge TSS concentrations from several sources.

Source	Sludge TSS concentration	Reference
Upflow sand filter	0.005-0.015%	Malone and Burden (1988)
Sand filtration	0.01-0.02%	Metcalf and Eddy (1979)
EGB	0.05-0.5%	Chen <i>et al.</i> (1993)
Primary sedimentation	1-6%	Chen <i>et al.</i> (1993)

Table 4. A comparison of sludge stabilization options.

Method	Advantages	Disadvantages
Anaerobic lagoon	High organic loading capacity Low maintenance	Odor
Aerated lagoon	High organic loading capacity Low area requirement	Large energy consumption Moderate maintenance
Composting	Useful end-product	Dewatering required Moderate capital expense
Anaerobic digester	High organic loading capacity Methane generation	Complicated management High maintenance

by the frequency, duration and vigor of the backwash. Higher sludge retention times (2-5 days) tend to encourage the biodegradation of solids concomitant with enhanced nitrification rates and decreased water losses. Increasing the settling time after a backwash is another means of significantly increasing sludge density. These factors are important to the linkage of internal and external sludge treatment processes.

High nitrogen contents (4-6%), phosphorus levels of about 2% and the absence of contaminants, such as heavy metals, make aquacultural sludge attractive as a fertilizer (Willett and Jakobsen 1986). Direct land application has proven feasible in areas with dry climates where the high moisture content of the sludge is considered beneficial. In wet climates, additional stabilization of sludge may be required to avoid odor and runoff problems. Table 4 lists some of the more common methods for digesting sludge, along with the important advantages and disadvantages of each method.

Anaerobic lagoons are inexpensive and easy to operate. However, because of their odor, they are seldom suited for any but the most remote locations. Aerated lagoons have an organic loading capacity similar to anaerobic lagoons with no offensive odor, but aeration equipment is initially expensive with continuing operations and maintenance costs. Composting yields soil conditioner, which is a commercially valuable end-product. However, composting requires mechanical dewatering prior to stacking in static piles or windrows, and static piles must be aerated while windrows require periodic mixing. Anaerobic digesters are very popular for stabilizing sludge from municipal waste treatment plants, but they are expensive and their management requires specialized knowledge of their microbiology.

SUMMARY

Recirculating aquaculture systems provide a means for

actively controlling the quality of the aquatic species being produced. RASs also mitigate the negative environmental impact caused by the continuous discharge of organic and mineral contaminants by large-scale aquaculture production systems. An integrated approach to solids treatment utilizes the synergy between internal and external processes to reduce costs. Manipulation of the bead-filter backwash regime can result in a substantial reduction in discharged sludge mass, through biodegradation, without adversely affecting nitrification. Dilute sludge produced by backwashing or washdown operations can be concentrated by internal settleline or by external clarification processes prior to stabilization and disposal. Aerobic and anaerobic processes with extensive track records are available to reduce the easily biodegradable portion of discharged sludge, minimizing the volume of sludge for final disposal. As a final disposal option, land application appears most feasible for rural areas, whereas landfilling may be most appropriate for urban areas.

In this paper, we have focused on solids discharge because it presents the greatest threat of environmental degradation, particularly oxygen depletion and destruction of benthic communities. However, the discharge of nutrients, i.e., nitrate and phosphorous, can cause eutrophication of the receiving water. The major problem associated with nutrient enrichment is an algal bloom, providing the basis for an oxygen crash when water quality or environmental conditions change. Nitrate removal can be accomplished by denitrification within or external to the RAS. If denitrification is provided within the RAS, some of the alkalinity lost in the nitrification process will be replenished. Phosphorous removal methods are expensive and the reformulating of aquatic feeds to increase metabolically available phosphorous is the most promising means of phosphate discharge reduction.

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Management of a Seawater Recirculation Fish Culture System for Japanese Flounder

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ABSTRACT

Rearing experiments of the Japanese flounder, *Paralichthys olivaceus*, were carried out under high fish density conditions using a closed seawater recirculation system of 22 m³ in total water volume. After 330 days of rearing, fish grew to 480 g in mean body weight (initial: 3.5 g) and total fish biomass in the system was 844 kg. Rearing density per unit volume of water in the system reached 38.4 kg/m³. Because 6 and 9 m³ of the rearing water exchanged with fresh seawater at the measurement of fish body weight, the product per unit volume of seawater used was 22.6 kg/m³. Ammonia and nitrite concentrations of the rearing water were maintained under 1 mg-N/L during the first 100 days; however, after that, ammonia concentrations fluctuated between 0.5 and 7.8 mg-N/L and nitrite between 1.0 and 4.0 mg-N/L. Nitrate concentrations increased with the cumulative amount of feed and reached 359 mg-N/L on the 190th day. Then they decreased from 312 to 120 mg-N/L during the period from the 220th to the 263rd day, because of the occurrence of denitrification resulting from the formation of local anaerobic areas in the system. Although there were some fluctuations of ammonia, nitrite and nitrate concentrations, these results indicate that intensive culture of Japanese flounder is possible with a small quantity of seawater without daily water exchange and without direct impact on the aquatic environment by using the closed seawater recirculation system.

INTRODUCTION

Most of the saltwater fish culture is managed with cages on the coast of Japan. Some kinds of fish, such as Japanese flounder, are reared in tanks built on land with flowing water pumped from the sea. This fish culture method is called the flow-through method. Because these two types of fish culture depend on the natural sea and seawater, fish growth and management of the systems are affected by seasonal changes, weather conditions and other factors. Moreover, these two open fish culture systems discharge feces of rearing fish and leftovers directly into the sea. Therefore in some areas, they cause deterioration of the culture ground and water pollution (Piedrahita 1994, Van Rijn 1996).

On the other hand, closed water recirculation fish culture systems have the advantage of using very small quantities of water for fish production compared with flow-through systems. Therefore, it is easier to maintain an optimum temperature for rearing species in the closed systems. Closed systems have another advantage of less direct impact on the aquatic environment than open systems when waste materials from the systems are managed properly.

Since 1986, we have been studying closed seawater recirculation systems for Japanese flounder from the view-

point of obtaining optimum conditions using electric power and also reducing the impact on the aquatic environment, Honda et al. (1991) reported on the possibilities of intensive culture of Japanese flounder without the wasteful use of seawater, with a closed seawater recirculation system. The present report deals with a rearing experiment of Japanese flounder using a large closed seawater recirculation system of 22 m³ in total water volume.

MATERIALS AND METHODS

RECIRCULATION SYSTEM AND ITS OPERATION

The system was planned to produce 2,000 fish of 500 g in body weight, which is the minimum commercial size for cultured flounder in Japan. The system consisted of a fish tank, a settling tank, two biological filters, a heating-cooling unit, a circulation pump, a UV light unit and three air-blowers. The minimum necessities of filter media, aerator capacity, seawater for operation and bottom area of fish tank were calculated based on the results of other experiments, such as upper rearing density, respiration rate, ammonia excretion rate of fish and ammonia oxidation rate of biological filters (Honda 1988, Honda et al. 1991, Kikuchi et al. 1990, 1991, 1992, 1994) as shown in Fig. 1. The schematic diagram of the system is shown in Fig. 2.

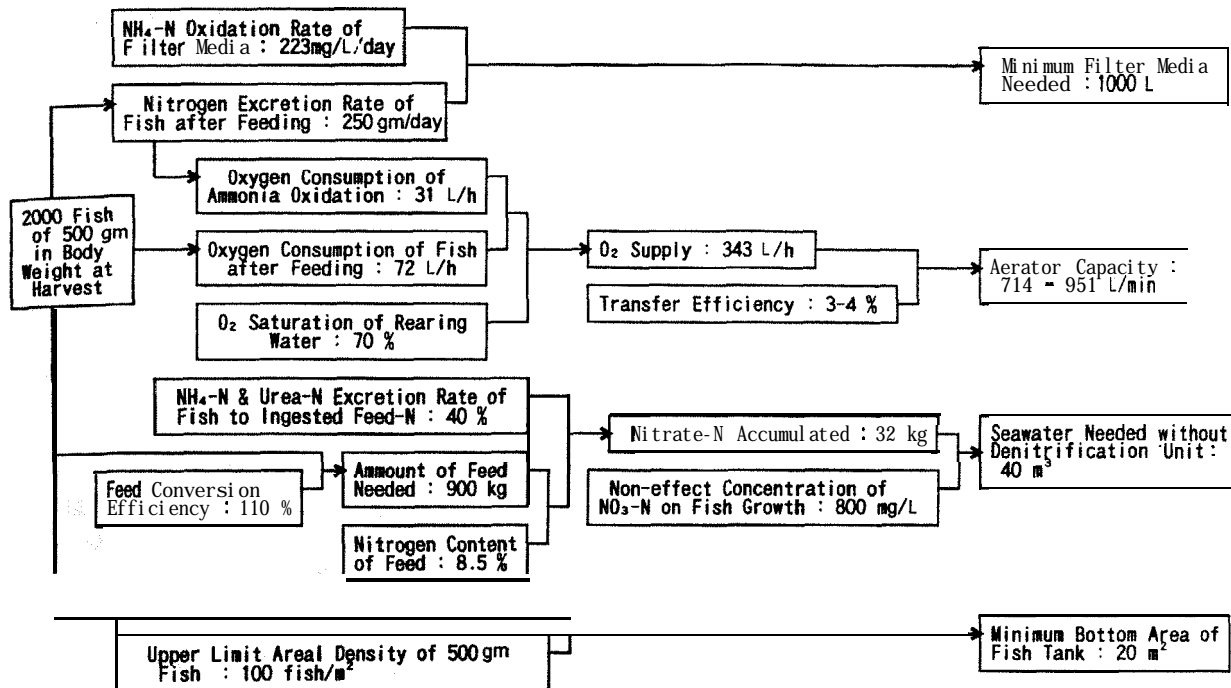


Fig. 1. Outline of design process of closed systems for Japanese flounder.

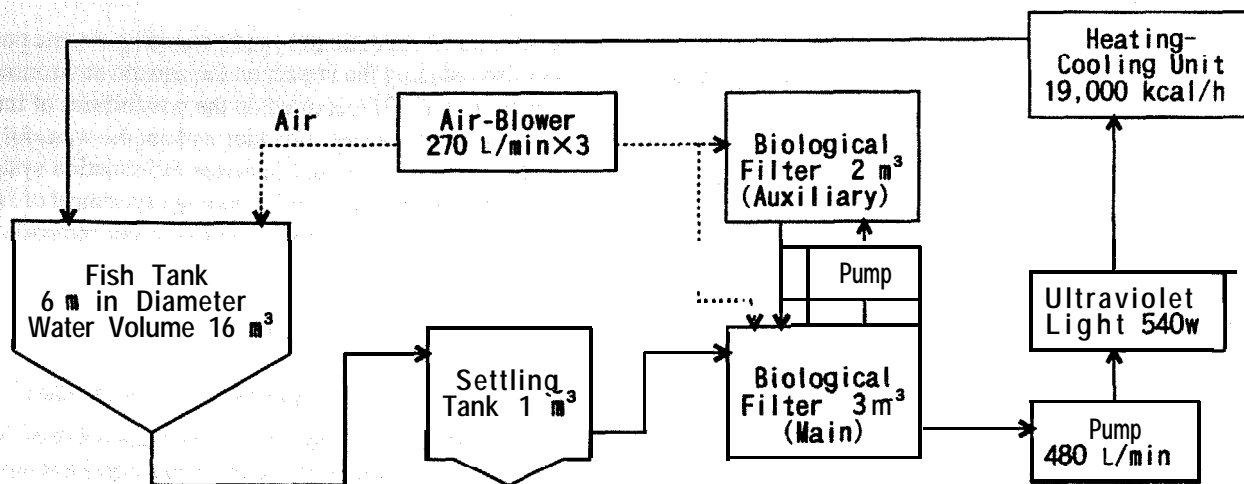


Fig. 2. Schematic diagram of the closed seawater recirculation system.

The fish tank was 6 m in diameter and the bottom sloped to 0.1 m in the center. Average water depth was 0.6 m and the water flow rate at the inlet was adjusted to 0.4 m³/min. The biological filters consisted of a 3-m³ main filter tank and a 2-m³ auxiliary filter tank connected in bypass with a pump of 0.1 m³/min. Filter media of 1.3 m³ of small net-type plastic, which have a specific area of ca. 350m²/m³ and were previously well conditioned, were placed in the main filter tank, and 0.5 m³ of filter media were also placed in the auxiliary tank. A submersible water pump with a capacity of 0.1 m³/min was placed in the main biological filter tank on the 265th day to circulate water in the tank sufficiently. The settling tank of 1 m³ was equipped with the system just before the main biological filter tank. To determine the production rate of sediment in the settling tank and biological filters, sediment was not removed from the tanks during the first 115 days. After that, sediment in the tanks was removed every two months. Natural seawater used in this experiment was collected at the coast near Onjuku, Chiba Prefecture. Because the optimum temperature for the growth of the flounder is 20 to 25°C (Iwata et al. 1994), water temperature was controlled at 25°C in summer and 20°C in winter. The pH of the rearing water was maintained between 7.0 to 7.5 with NaHCO₃. To adjust the salinity of the rearing water, fresh well water was added corresponding to the evaporated volume. Dissolved oxygen of the rearing water in the system was maintained with air-blowers and air-stone diffusers placed in the fish tank and the biological filters. The numbers of blowers were increased from one to three with growth of the fish. The total water volume in the system was adjusted to 22 m³. Six and 9 m³ of rearing water were exchanged with fresh seawater at the time of measurement of fish body weight on the 193rd and 242nd days.

REARING OF FISH

On April 21, 1994, 2000 fish of 3.5 g in mean body weight, obtained from a hatchery in Mie Prefecture, were stocked in the fish tank at a density of 70 fish/m². The fish had been starved for 72 h before the measurement of body weight. The fish were fed until satiation on a commercial diet containing ca. 8.5% of nitrogen twice a day from Monday to Friday and once on Saturday and Sunday. The feedings totaled 313. The rearing continued until fish reached ca. 500 g in body weight. To estimate the increase of fish biomass, body weights of 50 to 150 fish were measured at ca. 30- to 40-day intervals. On the 242nd day and at the end of the experiment, body weights of all fish in the system were measured. The numbers and body weights of dead and removed fish were recorded to correct the nitrogen budget in the system.

ANALYSIS OF REARING WATER

Ammonia concentration was determined by the in-

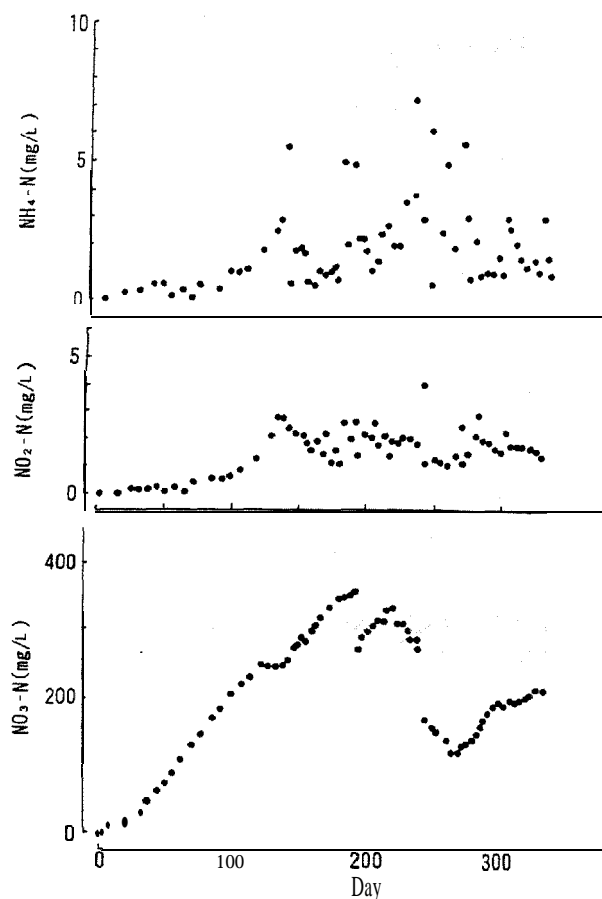


Fig. 3. Fluctuations of ammonia, nitrite and nitrate concentrations in the rearing water of Japanese flounder.

dophenol method. Nitrite concentration was determined by the Griess-Romijn method. Nitrate concentration was measured with an ion chromatographic analyzer (IC-500, Yokogawa Electric Co., Tokyo). Oxygen concentration was measured with a DO meter (YSI model 58, Yellow Spring Instruments Co. Inc., Yellow Spring). The pH was measured with a pH meter (M-8, Horiba Seisakusho Co., Kyoto). These concentrations were measured routinely twice a week, usually on Tuesday and Friday. Water for analysis was sampled in the morning before feeding.

RESULTS AND DISCUSSION

WATER QUALITY

Ammonia and nitrite concentrations of the rearing water were maintained under 1 mg-N/L during the first 100 days; however, after that, ammonia concentrations fluctuated between 0.5 to 7.8 mg-N/L and nitrite between 1.0 to 4.0 mg-N/L (Fig. 3). The mean concentrations of ammonia and nitrite were 1.95 ± 1.62 and 1.62 ± 0.88 mg-N/L (mean \pm SD), respectively. As shown in Fig. 3, nitrate concentrations increased with the cumulative amount of feed (see Fig. 7) and reached a maximum of 359 mg-N/L on

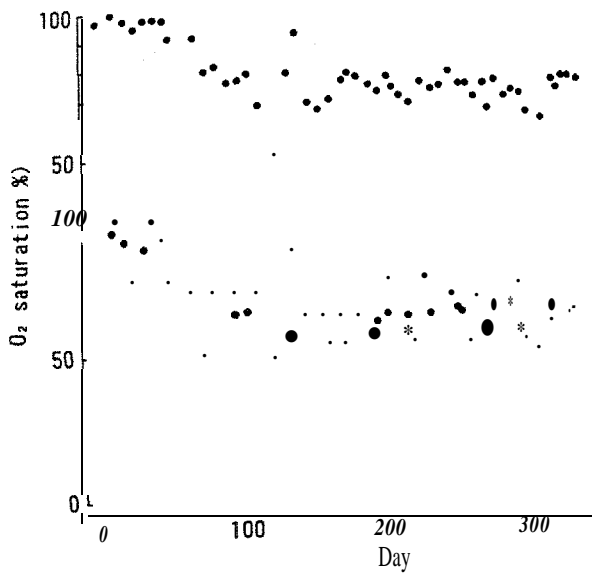


Fig. 4. Dissolved oxygen levels of rearing water in the fish tank (upper) and at the outlet of the main biological filter (lower).

the 190th day. After **exchange** of 6 m³ of water, nitrate concentration decreased to 277 mg-N/L on the 193rd day and then increased until 312 mg-N/L. Nitrate concentrations decreased to 120 mg-N/L during the period from the 220th to the 263rd day, because of the occurrence of denitrification resulting from the formation of local anaerobic areas in the system and the water exchange of 9m³ on the 242nd day. After the placement of a submersible water pump in the main filter tank on the 265th day, nitrate concentrations increased and reached 213 mg-N/L, and fluctuations in ammonia concentrations became smaller. From these facts, it was concluded that fluctuations of ammonia, nitrite and nitrate concentrations resulted from insufficient circulation of water in the biological filter tank.

Dissolved oxygen levels in the rearing water in the fish tank were more than 70% of saturation through most of the experimental period. After the 100th day of rearing, levels of dissolved oxygen measured at the outlet of the main biological filter often decreased to 50% as shown in Fig. 4. From these facts, it is possible that some local anaerobic areas less than 30% in saturation levels were formed in the biological filter and brought about denitrification.

The total amount of NaHCO₃ added to the rearing water for pH adjustment was 65 kg. The total volume of well

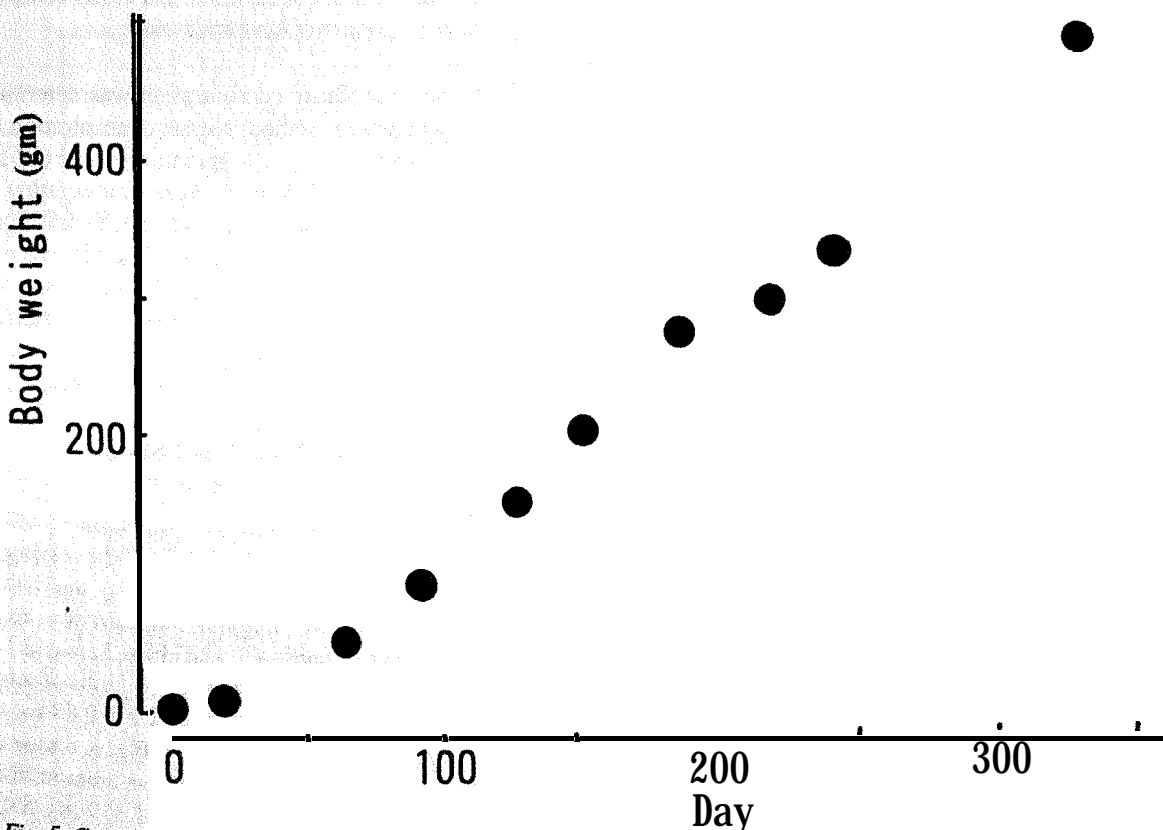


Fig. 5. Growth curve of Japanese flounder. Body weight: mean body weight/fish.

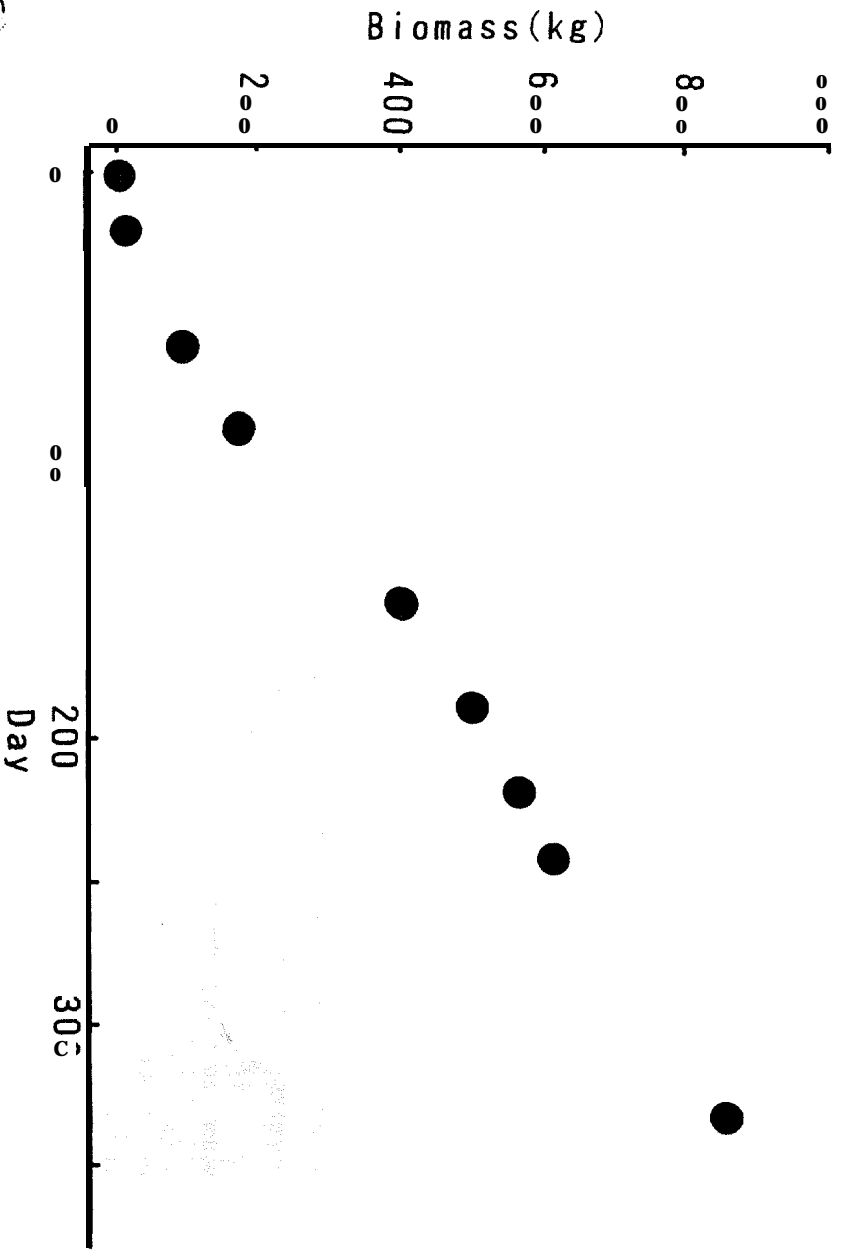


Fig. 6. Increase of biomass in the fish tank.

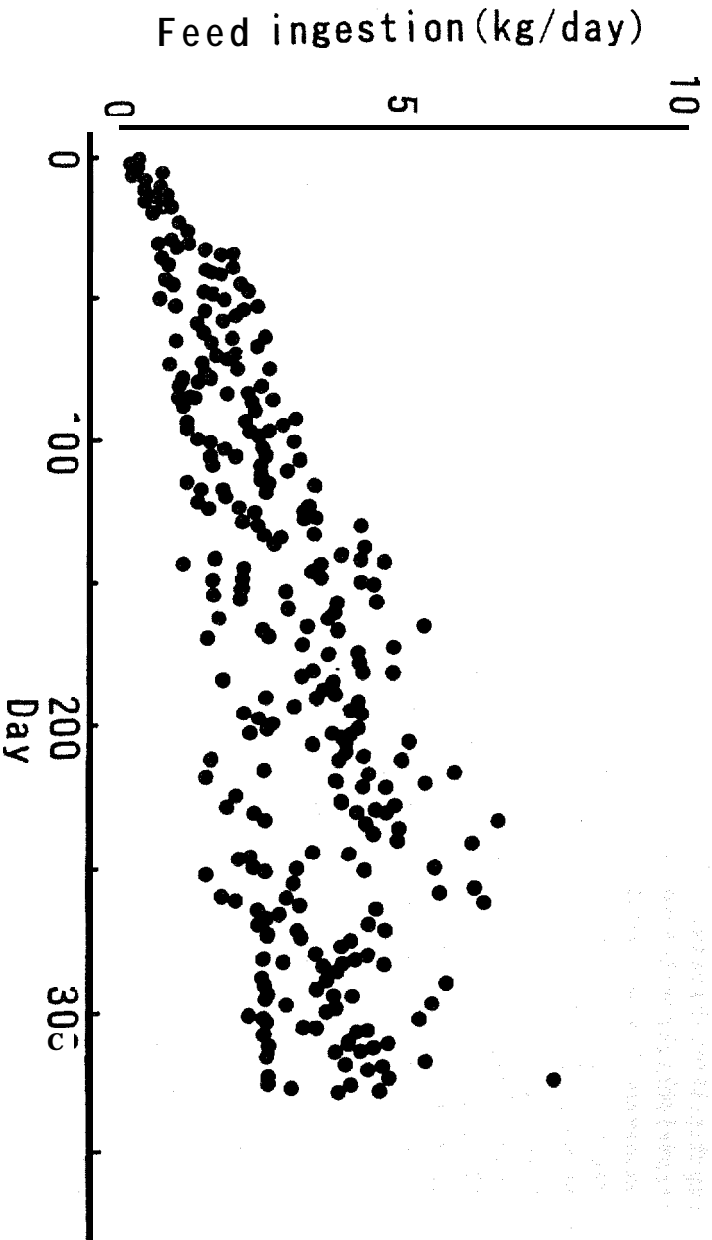


Fig. 7. Daily feed ingestion of Japanese flounder.

Table 1. Results of 330 days rearing of Japanese flounder.

Survival rate(%)	88.0
Final biomass(kg)	844.3
Weight increment(kg)	837.3
Feed ingested(kg)	842.0
Feed conversion efficiency(%) ^a	99.5
Density per unit volume of water(kg/m ³)	38.4
Density per unit area of fish tank(kg/m ²)	30.1
Production per amount of water used(kg/m ³)	22.6
^a FCE: Fish in wet/ feed in almost dry	

water used to adjust the salinity was 10.3 m³. Concentrations of suspended solids in the rearing water measured occasionally during the operation were determined to be almost 3 to 5 mg/L, although the concentrations increased from 10 to 11 mg/L just after feeding.

FISH GROWTH

Although high concentrations of ammonia and nitrite were sometimes observed, the fish grew normally compared with our past experimental results and reached 480 g in mean body weight after 330 days rearing, as shown in Fig. 5. The total fish biomass in the fish tank reached 844.3 kg (Fig. 6). As shown in Fig. 7, daily feed ingestion increased with fish growth and a maximum of 7.7 kg/day was observed on the 306th day. It was estimated that ca. 260 g of ammonia was excreted. The survival rate was 88% and the total weight increment was 837.3 kg. Total feed weight ingested by fish was 842 kg, so feed conversion efficiency (fish: wet/feed: almost dry) became 99.5%. The corrected feed conversion efficiency added to the weight of dead and removed fish was ca 110%. The figures of feed conversion efficiencies were almost equal to our past experimental results and the efficiencies of juvenile Japanese flounder reared by flow-through systems (Saitoh *et al.* 1990). The fish density per unit volume of water in the system reached 38.4 kg/m³ and areal density in the fish tank was 30.1 kg/m². Since the weight increment was 837.3 kg and the total amount of seawater used for rearing was 37 m³, the product per unit volume of seawater used reached 22.6 kg/m³ (Table 1). This result implies that 1 kg of flounder was produced in only 44 L of seawater

NITROGEN BUDGET

Nitrogen budget in the system was estimated based on the total feed weight ingested, the weight increment and

the total weight of dead and removed fish from the system. In this estimation, the nitrogen content of feed was 8.5% and fish was 3.7% based on our analyses. As shown in Fig. 8, about half (33.8 kg, 47.3%) of the nitrogen contained in the feed (71.5 kg, 100%) ingested by fish was transferred into fish protein, and another half (37.7 kg, 52.7%) was excreted from the fish into the rearing water. This budget agreed with an earlier determination of nitrogen excretion rate of the flounder (Kikuchi *et al.* 1991), although the proportion of excreted nitrogen was slightly higher in this study. On the basis of the nitrogen excretion rate of the flounder, excreted ammonia, urea and feces were estimated at 27.5 kg (38.5%), 3.8 kg (5.3%) and 6.4 kg (8.9%), respectively. Nitrate accumulated in the rearing water was estimated at 31.3 kg (43.8%) when all of the ammonia and urea excreted from the fish were oxidized to nitrate. About 70% (21.9 kg) of the nitrate accumulated was removed from the rearing water by denitrification. Feces nitrogen was not traced completely in this study. The sediment in the settling tank and the biological filter tanks was collected on the 115th day. The total weight of the sediment was 5.2 kg dry weight. This weight was ca. 1/7 of the estimated feces weight based on total feed weight ingested during the first 115 days and the nitrogen excretion rate of the flounder. Possibly a substantial amount of organic matter in the feces was decomposed and the inorganic nitrogen was released into the rearing water. In the settling tank, a large number of polychaetes (*Capitella* sp.) appeared around the 200th day of operation. The results of our preliminary experiments showed that an individual polychaete ingested 0.7 mg (dry weight) of the feces of Japanese flounder (33.7 mg-N/gm) and excreted 0.27 mg of feces (25.4 mg-N/gm)/day at 25°C (Fig. 9). In this process, ca 70% of the nitrogen included in the feces of the flounder was reduced. From these results, it is considered that a large part of the nitrogen in the feces of the flounder was transferred to the polychaete during the last 130 days.

OPERATION COST

In the 330 days of operation, 2000 seedling fish, 842 kg of feed and 41,237 kWh of electricity were used to produce ca. 840 kg of flounder. Unit prices of these items were 120 Japanese yen/fish, 350 yen/kg of feed and 11 yen/kWh, respectively. Therefore, the cost for 1 kg production of the flounder was 1180 yen (ca US \$11). This is not a great difference from the total cost of seedling, feed and electricity in the current flounder culture with open flow-through systems, notwithstanding the operation of a heating-cooling unit to keep the optimum temperature for growth of the flounder. The cost of the closed sea-water recirculation system used in this study was 1.5 to 2 times higher, however, compared with the flow-through systems consisting of 6-m diameter fish tanks and other systems.

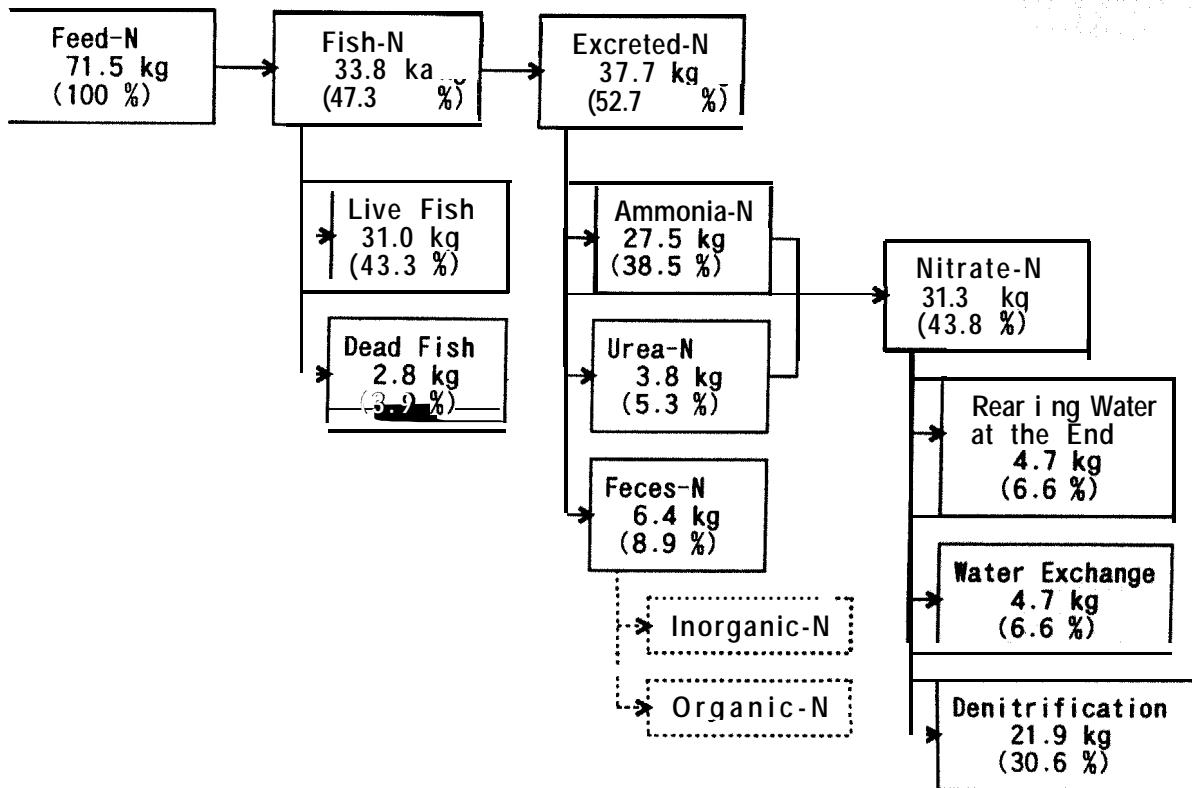


Fig. 8. Estimated nitrogen budget in the system during 330-day operation.

CONCLUSION

The results of this rearing experiment demonstrate that intensive culture of Japanese flounder is possible with a small quantity of seawater, without daily water exchange and without direct impacts on the aquatic environment by using a large closed seawater recirculation system, although there is some need of an economic feasibility study on the commercial operation of the system.

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