Preface

The United States and Japanese counterpart Panels on Aquaculture were formed in 1969 under the United States-Japan Cooperative Program in Natural Resources (UJNR). The UJNR Aquaculture Panel currently includes specialists drawn from the government and academic departments most concerned with aquaculture. Charged with exploring and developing bilateral cooperation, the Panels have focused its efforts on exchanging information related to aquaculture that could be of benefit to both countries.

The UJNR was begun during the Third Cabinet-Level Meeting of the Joint United States-Japan Committee on Trade and Economic Affairs in January 1964. In addition to the aquaculture Panel, current subjects in the program include destination of seawater, toxic microorganisms, air pollution, energy, forage crops, national park management, mycoplasmosis, wind and seismic effects, protein resources, forestry, and several joint Panels and committees in marine resources research, development, and utilization.

Accomplishments of Aquaculture Panel include: increased communication and cooperation among technical specialists; exchanges of scientists and students; focus of efforts to issues of major international concerns such as disease transmission and stock enhancement; exchanging of information, data, and research findings; annual meetings of the Panel; administrative staff meetings; exchanges of equipment, materials, and samples; several major technical conferences; and beneficial effects on international relations.

The 31st UJNR Aquaculture Panel Business Meeting and Symposium was held from October 16 to October 17, 2002 at National Research Institute of Fisheries Science in Yokohama, Japan. The first topic of the sixth five-year plan of the UJNR Aquaculture Panel from 2003 to 2007, "*Aquaculture and Stock Enhancement of Algae and Filter Feeders*", provided the basis and focus for the Symposium. Following the meeting and symposium, field trips and satellite symposium were held in Shizuoka Prefecture, central Japan and Miyagi Prefecture, northern Japan to visit prefectural fisheries research organizations, culture sites of seaweeds and bivalves from October 18 to 24, 2002.

The symposium was organized by representative coordinator Dr. Tetsuo Seki and other UJNR Aquaculture Panel staff members of the Japanese side. Twenty specific papers were presented in the symposium. Eighteen full papers of them have been accepted for this Proceedings as a Supplement of Bulletin of Fisheries Research Agency. Two abstracts were included in this Proceedings. Editorial work has been assisted by the staffs of National Research Institute of Aquaculture. We would like to acknowledge Dr. Hisashi Kurokura, Dr. Ken Furuya, Dr. Charles Yarish and Dr. Roger I. E. Newell to preparing keynote and identifying speakers, and Dr. Masao Ohno, Kochi University for presiding the general discussion. On behalf of Japanese authors, we would like to express sincere gratitude to Dr. Paul Kilho Park for revising English with great dedication. Finally we wish thank Ms. Maya Kurihara to greatly support a editorial task for this Proceedings.

Chairman:
Yasuji Sakai, Japan
James P. McVey, United States

水研センター研報，別冊第１号，１－４，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 1-4, 2004

The importance of seaweeds and shellfishes in Japan: Present status and history

Hisashi KUROKURA*

Abstract Though many manufactured goods are produced from aquatic resources, people living in Japan consume them mainly as food. Among such sea foods, seaweeds and shellfishes have special religious meaning. Kombu (*Laminaria*), wakame (*Undaria*) and abalone or other shellfishes are commonly included in offerings to Shinto shrine. It was supposed from this fact that these foods were important for Japanese ancestor. In fact, many remains of seaweeds and shells were dug out from ruins of prehistoric age. In the 8th century, Yakamochi Ohtomo, a famous poet in Nara Period, transplanted a shell species from Kishyu to Ecchyu. This is the oldest record of propagation of shellfish. Nori (*Porphyra*) is now indispensable for sushi. Its culture started from the 17th century. Aquaculture of wakeme, oyster, peal oyster, and many other seaweeds and shellfishes had begun by the end of 19th century. Aquaculture technology has been improved and the production has increased rapidly in the latter half of last century. Now seaweeds and shellfishes aquaculture encounter various difficult problems such as changes in coastal environment by pollution and land reclamation. On the other hand, the function of these aquaculture for water purification attracts attentions of people together with the increase of interest to environment issues.

Key words: shellfishes, seaweeds, Aquaculture, history

Importance of Seaweeds and Shellfishes in Japanese Daily Life

In 2000, the average family income and expenditure for consumption were 6,371,000 yen/year and 3,806,000 yen/year in Japan (Ministry of Public Management, Home Affairs, Posts and Telecomminucations, Japan, 2001). Within the expenditure for consumption, 972,000 yen/year was consumed for foods including 174,000 yen/year for dining out (Fig. 1). Total amount of the expenditure for livestock products, such as beef, pork, chicken, including hum, sausage, milk, and egg was 119,000 yen/year, and same money (118,000 yen/year) was paid for fresh aquatic food. Besides the expenditure for fresh aquatic foods, 49,000 yen was consumed for processed aquatic food, such as dried fish and "surimi" products. From these data, it can be supposed that an average Japanese family pays 1.5 times larger amount of money for aquatic foods than livestock products, and that the expenditure for aquatic foods reaches 21% of total expenditure for foods excluding dining out (Fig. 2).

In expenditure for fresh aquatic foods, more than half of it was occupied by fin fish (67,000 yen/year) and major parts of the remainder were expenditures for crustacean and cephalopod. Only one tenth of the expenditure for finfishes was paid for shellfishes (6,400 yen/year). "Asari" (Manila clam, *Ruditapes philippinarum*), Pacific giant oyster (*Crassostrea gigas*) and scallop (*Patinopecten yessoensis*) are

2003年６月12日受理（Received on June 12, 2003)
* Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, 113-8657, Japan

main shellfishes consumed in Japan. Nori (*Porphyra spp.*), konbu(*Lminaria spp.*) and wakame (*Undaria pinnatifida.*) are major seaweeds eaten in Japan. They are consumed as processed aquatic food (dried, salted, or boiled etc.) and the total amount of expenditure for seaweeds (6,200 yen/year) was same as that for shellfishes (Fig. 3).

In protein base, daily protein intake by Japanese was 86.8g/day/individual in 2000 (Minister's Secretariat, Ministry of Agriculture, Forestry and Fisheries, Japan, 2001). Within 86.8g of daily protein intake, 20.9g and 7.5g were from cereals and beans. Live stock products provided 28.4g of protein. Within that, 14.4g, 5.7g and 8.3g were by meats, eggs and milk and milk products, respectively. Total volume of protein supplied by aquatic animal including fish, molluscs, crustaceans and echinoderms was 19.4g. Only 1.1g was supplied from seaweeds (Fig. 4).

Though the contributions in the family expenditure and protein supply are not so high when compared with fishes and squids, shellfishes and seaweeds are very important food materials for Japanese cuisine. They provide delicate taste, flavor and trace nutrients to the cuisine. For example, "sushi" can not be completed without these materials. The notable importance of shellfishes and seaweeds are supported by Japanese environment and history.

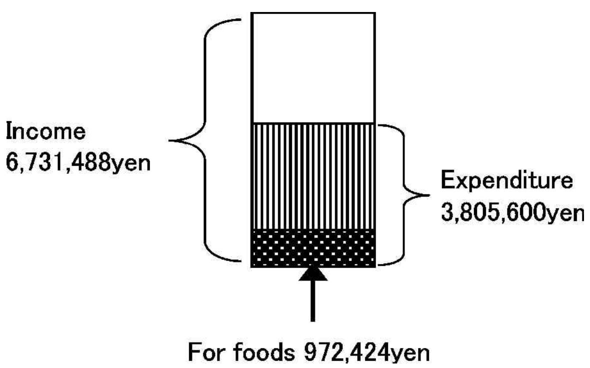

Fig. 1. Average family income, expenditure and expenditure for foods in Japan in 2000

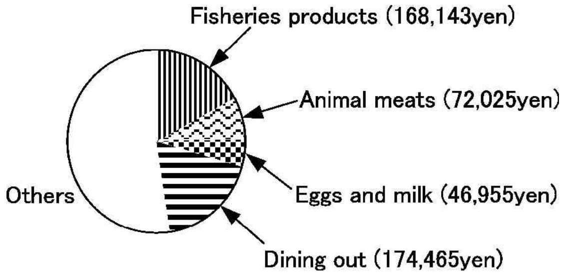

Fig. 2. Expenditure for each food item in 2000

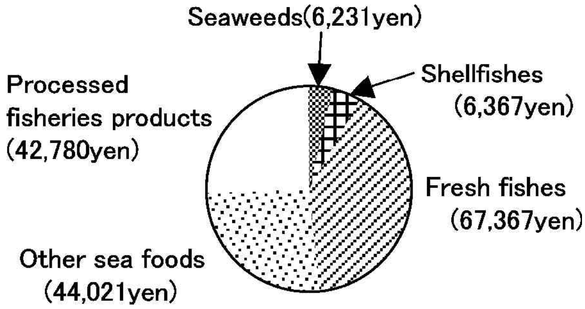

Fig. 3. Expenditure for fisheries products in 2000

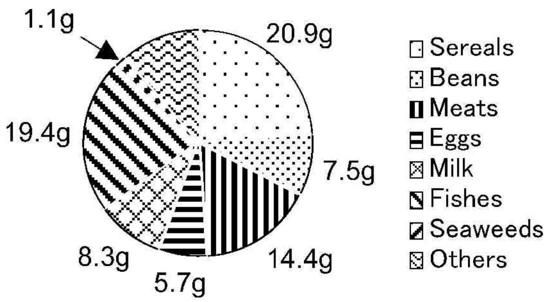

Fig. 4. Protein intake of average Japanese (g/individuals /day) in 2000

History of Aquaculture of Shellfishes and Seaweeds

Shinto is basically ancestor worship in Japan, and the offerings in Shinto shrines originally were dinner of the ancestors. The other side of the offering is promotion of local industries and they have been modernized and standardized twice in modern era. However, several old shrines such as Kamo Shrine and Ise Shrine keep old style in their offerings. We can find abalone, hard clam and other shellfishes and various kind of seaweeds in those offerings. This fact shows Japanese have been eating shellfishes and seaweed as daily food from prehistoric age.

In 764, Yakamochi Ohotomo, a poet in the Nara period, the last editor of Manyohshu, transplanted a species of bivalve from Kishyu (Pacific coast of Honshu island) to Ecchyu (Sea of Japan side). This is the oldest record of transplantation of bivalve and attempt for propagation of aquatic animal. First record of artificial propagation of seaweed was also transplantation. In 1081, a kind of seaweed was transplanted from Kouzu-shima, an island in Izu archipelago, to Izu peninsula. Nori and oyster culture were supposed to be started in 1670s. In 1892 Koukichi Mikimoto started pearl oyster culture. Oyster culturists began to use rafts for hanging culture in 1925. Floating net culture of nori started in 1934 in Tokyo bay and other modern "nori" culture techniques, such as artificial spat collection, and cryopreservation of nori seeds, were established in the 1950s and early 1960s. These modern techniques enabled expansion of area and season of nori culture. As the result, production of nori in Japan increased 40 times from 1940s to 1990s (Fig. 5) (Ministry of Agriculture, Forestry and Fisheries, 2001). On the other hand, pollution of costal environment became considerable from 1950s on because of rapid economic growth in Japan, and coastal aquacultures often sustained heavy damages (e.g. oil pollution in Tokyo bay in 1954, in Wakayama in 1955). In 1997, Ishaya Bay, an accessory bay of Ariake Bay, was closed by water gates for land reclamation. Nori production in Ariake Bay prominently decreased in the culture season of 2000. Several fishermens' cooperations in Ariake Bay claimed that the poor harvest of nori had been caused by alteration of environment of Ariake Bay which was brought on by the close of Isahaya Bay. After that, sustainability of coastal aquaculture has been thought to be an indicator of health of coastal environment (Table 1).

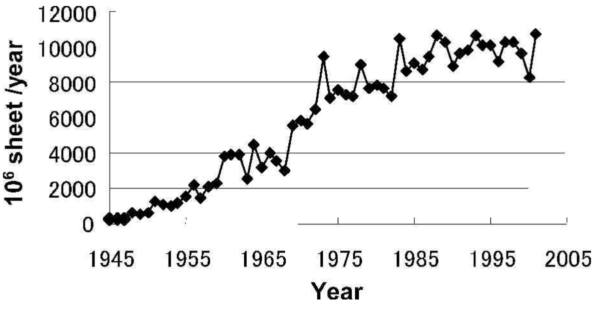

Fig. 5. Changes in production of Nori

Contemporary Importance of Shellfish and Seaweed Culture in Japan

Through the discussions about the relationship between the alteration of coastal environment and fisheries production, people understand the importance of aquaculture as a monitoring system of coastal environment and functions of shellfish and seaweed culture on the conservation of coastal environment. Fuyou Kaiyou Kaihatsu Co. Ltd. estimated the function of fisheries of asari on the environment in Hamana Lake, a brackish lake on the Pacific coast of Honshu Island. Total influx of carbon, nitrogen and phosphate to Hamana Lake, were 2,411, 1809 and 899 t/year respectively, and total catch of asari was 2,200 t/year in 1990. When we calculate the magnitude of the catch to the values based on carbon nitrogen and phosphate, they are 572, 132, 8.8 t/year. These magnitude mean that 26, 7.9, 1.3 % of carbon, nitrogen and phosphate introduced to Hamana Lake were removed by catch of asari. Similar effect can be expected to shellfish and seaweed culture. Now Japanese people has begun to notice the function of shellfish and seaweed culture on the conservation of coastal environment and they expect systematic approaches to utilize aquaculture for conservation of coastal environment.

Table 1. History of shellfish and seaweeds culture in Japan

year	
764	Transplantation of bivalve by Yakamochi Ohtomo
1081	First record of transplantation of seaweeds
1596	Name of "Asakusanori" began to be used for Nori harvested in Tokyo-bay.
1615	Transplantation of oyster from Wakayama to Hirosima
1674	Beginning of oyster culture
1673-81	Beginning of nori culture
1781	Transplantation of abalone from Bousou to Shrahama
1853	Use of bamboo stick as structure for nori culture
1870	Beginning of nori culture in Kanagawa
1878	Transplantation of konbu from Hokkaido to Aomori
1881	Beginning of asari culture in Chiba
1882	Beginning of nori culture in Aichi
1890	Koukichi Mikimoto began pearl oyster culture
1900	Damage of pearl oyster by toxic algal bloom *Gonyaulax* in Ago Bay
1912	Cleaning of rocky shore for the propagation of seaweeds
1920	Nets were firstly used as the substrata for nori attachment
1925	Start of hanging oyster culture
1926	Success in using net as the substrata of nori
1934	Floating culture of nori using floating net started in Tokyo Bay
1936	Beginning of spat collection of scallop in Lake Saroma
1942	Asari poisoned in Hamana Lake
1942	Success in artificial seed collection of wakame
1949	Discover of conochocelis Success in artificial seed collection of konbu
1954	Success in artificial seed collection of nori Leakage of heavy oil in Tokyo bay. (Nori culture was damaged)
1955	Nori culture in Wakayama was damaged by waste oil
1960	Mass mortality of cultured oyster in Hirosima bay
1963	Invention of freezing preservation method of nori seed
1964	Invention of spat collection method of scallop
1974	Success in artificial inducement of spawning of abalone by UV radiation
1997	Isahaya Bay, an accessory bay of Ariake Bay, was closed by water gate for land reclamation.
2000	Production of nori in Ariake Bay prominently decreased. The relationship between aquaculture production and alteration of costal environment became a national dispute

Cited from Ohshima, Y. (1994) with several modification by author

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水研センター研報，別冊第１号，５－12，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 5-12, 2004

The present situation and problems of oyster culture in Hiroshima Bay

Yasushi HIRATA* and Satoru AKASHIGE*

Abstract Hiroshima Bay including its adjacent area is one of the most important oysters farming areas in Japan. During 1980s, the oyster production in Hiroshima Bay was about 30,000 metric tons (t) scale by fresh meat weight (FMW) a year. In the early 1990s, the oyster production began to decrease and was about 20,000t in 2000. We analyzed the present situation and problems of oyster culture in Hiroshima Bay to show the problem solution. This decrease in oyster production in the 1990s was caused directly by typhoon damages; shellfish poisoning by *Alexandrium* spp.; and mortality by harmful dinoflagellate *Heterocapsa circularisquama*: and was caused by dense cultivation indirectly. The postponing of harvesting the oyster caused by shellfish poisoning prolonged the oyster culture period. The prolongation of the culture period in a limited culture ground caused eventually dense cultivation of oyster. Aged and large-sized oysters took much feed, therefore growth of all oysters in culture grounds were slowed down under low feed level. The low growth rate accelerated prolongation of a culture period. These vicious spirals promoted dense cultivation, and changed the environment of the culture ground into favorable environment for *H. circularisquama*. To analyze these situations, an oyster culture process model was built. This model showed that the reduction in oyster biomass in the culture grounds is needed to escape from the vicious spiral, and shortening of the culture period was the most effective method for this reduction without reducing harvest magnitude.

Key words: oysters, Hiroshima Bay, culture process

Introduction

We present a model to describe the difficulty that oyster culture in Hiroshima Bay including its adjacent area is facing currently. We have examined possible improvement of the culture process by this model.

Hiroshima Bay is located in the western part of the Seto Inland Sea (Fig.1). Oyster culture in the bay had begun at a tidal flat of estuary before 450 years ago. About 60% of a Japanese oyster are produced currently in Hiroshima Bay. The oyster hanging culture method by rafts of bamboo spread over the Hiroshima Bay after 1960. Kusuki (1991) had reviewed the history and technology of oyster culture in Japan.

The changes in annual oyster production by fresh meat weight (FMW) in Hiroshima prefecture after 1965 is shown in Fig. 2. The Hiroshima Fisheries Experimental Station (2001a) reported the relationship between the changes in oyster production and oyster culture technology as follows. In 1960s, the oyster production increased to 30,000t by spread of the hanging raft culture. However, in 1969 and 1970, the oyster production decreased by outbreak of red tide and dense settlement of serpulid worm (Arakawa, 1971). In 1970s, the volume of production increased to 30,000t

2003年６月12日受理（Received on June 12, 2003)
* Hiroshima Prefectural Fisheries Experimental Station, Ondo, Aki, Hiroshima, 737-1207, Japan

again. The recovery of production was achieved by development of a culture technique: hanging down deeply and migration of a raft seasonally, to avoid the red tide and the settlement of the other periphyton. In 1980s, improvement of the culture technology provided both reliable production of 30,000 t scale and the extension of a culture period. In 1990s, the oyster production began to decrease. In September 1991, a severe typhoon damaged 40% of the rafts, and the harvest was about 1 month late. In the early spring of the next year, harvest was stopped because a shellfish poisoning had exceeded safety regulation. The harvest suspension by shellfish poisoning in early spring occurred afterwards for several years. In addition, red tide of *H. circularisquama* occurred in autumn 1995 (Matsuyama *et al.*, 1997), and it delayed the oyster to gain weight. In summer 1998, mass mortality occurred by the red tide of *H. circularisquama*. In the next harvest season the oyster production was decreased to about 16,000 t. However, the oyster survived grew well. The Hiroshima Fsheries Experimental Station (2001a) estimated that the decreasing of oyster production after 1990 was caused by shortening of a harvest period by a shellfish poisoning and delay of growth or mortality by *H. circularisquama* directly. However, they thought that there were problems of production structure as arranging a lot of rafts in a limited culture ground and extending a culture period.

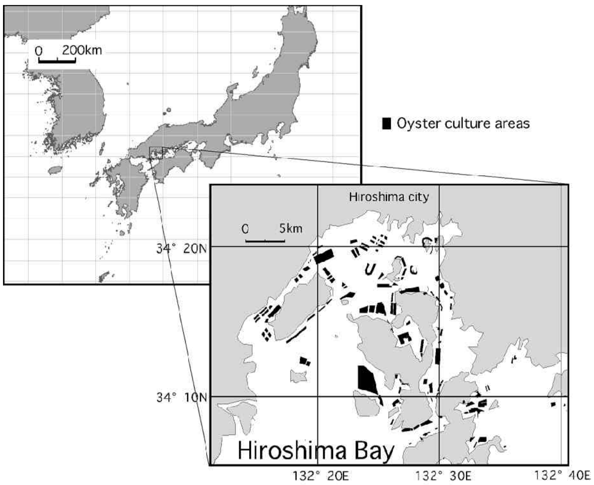

Fig. 1. Location of oyster culture areas in Hiroshima Bay

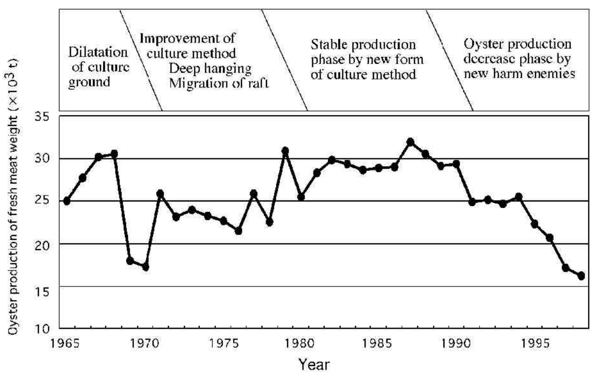

Fig. 2. Changes in cultured oyster annual production by fresh meat weight (FMW) in Hiroshima Prefecture. The data listed here can found in the "Annual Report of Production on Fishery and Aquaculture, Statistics and Survey Division", Ministry of Agriculture, Forestry, and Fishery, Japan.

In late years, studies of shellfish poisoning, red tide plankton, and that about evaluation of contribution degree of oyster culture in material circulation of Hiroshima Bay are pushed forward (Songsangjinda *et al.*, 1999). However, there are no concrete measures to elucidate their effects on oyster culture. In this study, we describe an approach to analyze the oyster culture methods in Hiroshima Bay by an oyster culture process model.

Materials and Methods

Oyster culture process model

1. Basic structure of the model

This oyster culture process model consists of physiological changes of an oyster and culture process of a raft unit in one culture ground. For modeling of feed competition among oyster, the structure that the quantity of feed in the culture ground decreases according to the biomass increases was built in this model. The calculation was carried out by one-day unit during one year from 1 October to 30 September. The relationship of increase in weight of an oyster and a quantity of chlorophyll in the culture ground, the relation of a size of oyster and a quantity of spawning eggs or death rate are determined by the findings of Hiroshima Fisheries Experimental Station (2001b).

2. Individual scale

Oyster repeats physiological change of gametogenesis and growth seasonally. In winter, oyster grows well. In summer; however, spawning reduces the weight. On 30 September, FMW for individual starts from 1g. The weight of oyster in this paper is expressed by FMW. The weight gain a day (*WG*) of an oyster from October to May was expressed by following equation:

$$WG=0.0512QC-0.0130,$$

where *QC* is the quantity of chlorophyll (μ g/L). The *QC* decreases by an oyster biomass (*OB*) in a culture ground. Environmental factors as water temperature, salinity and dissolved oxygen are not considered. The weight does not change in June and September, and decreases according to weight of June by spawning in August from July. Starting weight of September (*Ws*) is given by

$$Ws=-0.02\ Wjun^2+Wjun \qquad (Wjun \leqq 30\text{g}),$$

$$Ws=0.4\ Wjun \qquad (Wjun>30\text{g}),$$

where *Wjun* is the weight of June.

3. Raft scale

Initial population is 250,000 oysters a raft. Some oysters die in July and August. Number of September (*Ns*) is given by

$$Ns=Njun \qquad (Wjun<10\text{g}),$$

$$Ns=Njun \quad \{1-(Wjun/90)\} \ (10\text{g} \leqq Wjun<90),$$

where *Njun* and *Wjun* are number of June and weight of June, respectively.

4. Culture ground scale

Quantity of chlorophyll (QC μ g/L) in culture ground is constant, however, when biomass of oyster (*OB*) exceeds a fixed quantity, it decreases by competition. *QC* is given by

$$QC=2.7 \qquad (OB<14{,}000\text{t}),$$

$$QC=37{,}800/OB \qquad (OB \geqq 14{,}000\text{t}).$$

5. Harvest

Oysters more than 5 g (FMW) are harvested. The raft that contains the heaviest oyster in culture ground is harvested sequentially. Total of 6,300 rafts are harvested in one year, with the speed of 28 rafts a day during 16 October to 28 May in this model. The oyster biomass (*OB*) is a total of weight of all individual of a culture ground. Gross production of the year is provided by summing daily harvest.

6. Production economy

Money earned in a day is calculated from gross weight of the oyster that harvested on one day and unit prices of the day. The Unit price of Hiroshima oyster is generally high until December, but tend to gradually fall from January. The change of unit price set it as follows. The price of oyster is 1,200 yen a kg from October to December. The unit price decreases by a rate of 5 yen/kg/day from 1,200 yen/kg during January to February, and 1 yen/kg/day from March to May. A gross production amount of money of the year is provided by a total of daily production sales.

Estimation of oyster production under different culture patterns

The basic pattern of oyster culture in Hiroshima Bay is classified into 3 patterns according to the length of the culture period. In the 1-year-culture method called "Waka", oyster spats are collected in the summer, grown in the winter, and harvested before the following summer (less than 12 months old). In the 2-year-culture methods called "Yokusei" and "Ikisu", oyster spats are hardened on intertidal racks for 2 to 3 months and 6 to 10 months, respectively, after spat collection in summer, grown under rafts over the next summer, and harvested during the following harvest season (13-23 months old). In the 3-year-culture method called "Nokoshi", oyster spats are hardened by the same method as in the "Yokusei" method, grown over two summers, and harvested during the following harvest season (25-35 months old). The combination of these culture methods in Hiroshima Bay had changed as follows: In the 1960s, oysters were produced by a combination of the 1 and 2-year-culture methods. Only a 2-year-culture method was used from the 1970s to the beginning of the 1980s. In the latter half of the 1980s, the 3-year-culture method has been introduced in addition to the 2-year-culture method, and its ratio has increased after the consecutive outbreak of shellfish poisoning (Fig. 3). We estimated oyster production by the number of rafts needed, the gross production, the total amount of money earned, oyster growth and oyster biomass in five patterns of a combination of culture methods. In patterns 1, 2, 3, 4 and 5, the oysters were cultured only by a 1-year-culture method, a 1-year-culture and 2-year-culture method, a 2-year- culture, a 2-year-culture and 3-year-culture, and by only a 3-year-culture, respectively.

Estimation of impact of shellfish poisoning on the oyster production

When the quantity of shellfish poison in oysters would exceed safety regulation, the oyster harvest in Hiroshima Bay would be halted. Oysters that aren't harvested would be left in the culture ground until the next harvest season beginning in October. Shellfish poisoning of oysters had occurred in Hiroshima Bay every spring from 1992 to 1998, except in the year 1994. The mass mortality of oysters caused by the *H. circularisquama* red tide occurred subsequently in summer, 1998 (Fig. 4). We evaluated the impact of the suspension of harvest caused by shellfish poisoning on oyster production by using this oyster culture process model, as follows: In the initial year, 90 % of the 2-year-cultured oysters and 10 % of the 3-year-cultured oysters would be harvested (year 0). In every spring during next five years, the harvest would be suspended during the final 20 days of the harvest period due to shellfish poisoning occurred (years 1-5). In the summer of the fifth year (year 5), the *H. circularisquama* red tide occurred and oyster mortality would be assumed 100 % and 50 % in case of the 3-year-cultured and the 2-year-cultured oysters, respectively.

Results

Estimation of oyster production under different culture patterns

Fig. 5 shows the simulated result on the number of raft needed, gross production, and total amount of money earned under each oyster culture pattern. These five culture patterns correspond to the historic changes in the culture methods used in Hiroshima Bay. The culture methods in the 1960s, and from the 1970s to 1980s and 1990s, are almost equivalent to patterns 1 or 2, 3, and 4, respectively. The shift in culture methods from pattern 1 to pattern 3

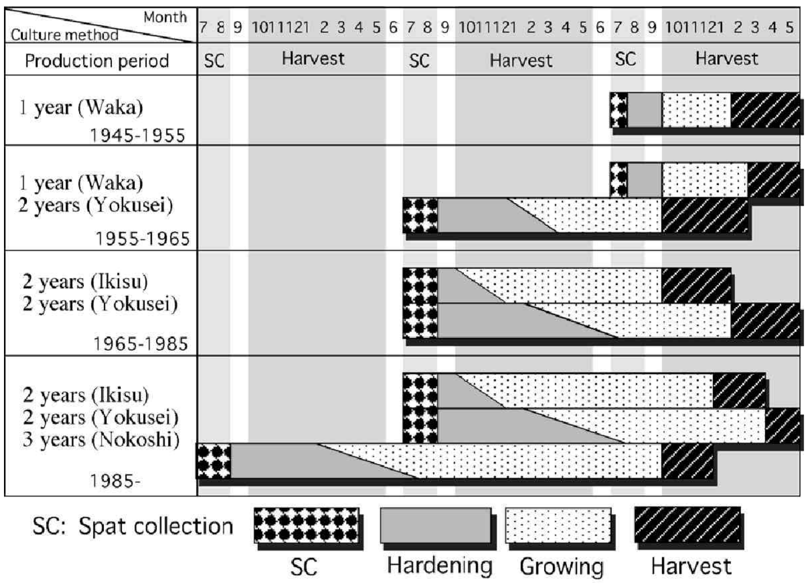

Fig. 3. Transition of oyster culture methods in Hiroshima Bay

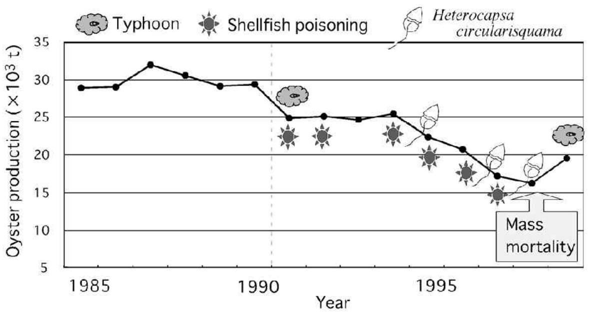

Fig. 4. Changes in annual cultured oyster production by fresh meat weight (FMW), and events which caused a production decrease in Hiroshima prefecture.

caused an increase of 12,600 in the number of rafts needed, which was twice the number used (6,300) in pattern 1. In pattern 3, both the gross production and the total amount of money earned was at its maximum. The shift in culture methods from pattern 3 to pattern 5 caused a further increase in the number of rafts needed, to 18,900 from 12,600. In patterns 4 or 5, the gross production and the total amount of money both decreased. Fig. 6a shows the changes of in the individual weight of harvested oysters in each harvest pattern. The shifts from pattern 1 to pattern 3 raised the oyster size of the harvest at the beginning, but reduced the oyster size of the harvest by the end of the exchange. The shifts from pattern 3 to pattern 4 raised the oyster size of the beginning harvest inconsiderably. However, the shifts from pattern 4 to pattern 5 reduced the oyster harvest size of all periods compared with pattern 3. A change of biomass under each culture pattern is shown in Fig. 6b. Fig. 6c shows the ratios of oyster age in each biomass of patterns 1, 3 and 5. The shift from pattern 1 to pattern 5 raised maximum biomass about 3 times.

Estimation of impact of shellfish poisoning on the oyster production

Fig. 7 illustrates the annual changes in the number of rafts and gross production on the condition that the mass mortality occurred after shortening of the harvest period by 20 days continued for 5 years. One year later, the gross production of oysters decreased because

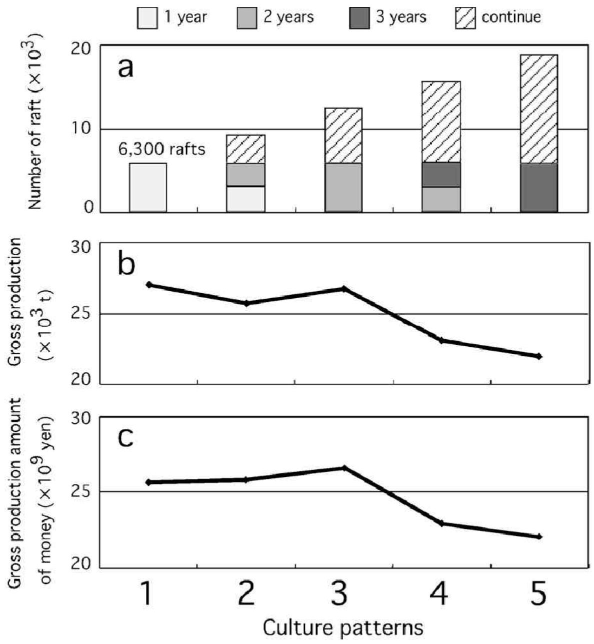

Fig. 5. The variation of: a) raft composition at the harvest of 6,300 rafts, b) estimated gross oyster production during each year, c) estimated total production of money in each culture pattern.

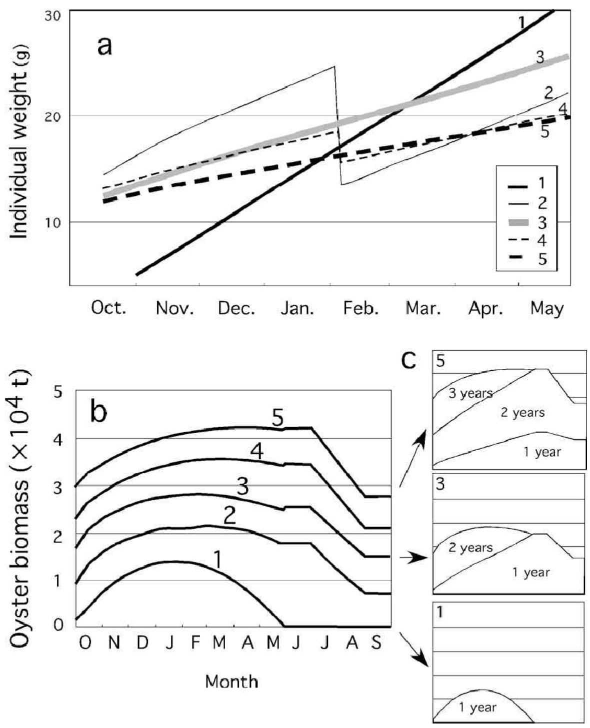

Fig. 6. Changes in the estimated: a) individual weight of oysters in each culture pattern, b) oyster biomass in each culture pattern, c) age composition of oysters in the biomass of patterns 1,3 and 5. The numbers in this figure correspond to each pattern in Fig. 5.

the harvest had been halted for 20 days, and the production decreased gradually thereafter. This gradual decrease in production was due to the postponement of the harvest schedule, which caused the increase in the ratio of the 3-year-culture, the change of the harvest size (Fig. 8a) and the increase in oyster biomass in the culture ground (Fig. 8b). Five years later, the harvest size in 1 December increased to 15.9 g from 14.9 g, but the harvest size in 1 May decreased to 19.8 g from 23.0 g (Fig. 8a). The increase of biomass for 5 years was remarkable in June, just before spawning. The biomass reached 1.34 times of the initial year (Fig. 8b). Due to the mass mortality which occurred in the summer of the 5th year, gross production in the 6th year decreased to about 16,000 t. However, individual oysters survived, showed high growth rate due to the decrease in the biomass (Fig. 8b).

Discussion

This oyster culture process model simulated the oyster culture production structure of Hiroshima Bay, reproducing each changes of the harvest size, gross production, and the total amount of money earned. This model enabled to obtain a long-term guide for selecting a culture method.

In the 1980s, oyster had been cultured mainly by the 2-year-culture method and the production had been constant approximately 30,000 t in Hiroshima Bay. But in this period, the 3-year-culture method was introduced to a small percentage and was effective because large size individuals were available to be harvested in the early period of harvest seasons, when the unit price was high. However, the induction of the 3-year-culture method caused an increase in the oyster biomass in the culture ground, delayed individual growth, and then prolonged the culture period, which led to a vicious spiral (Fig. 9). In the 1980s, we thought that the vicious spiral illustrated in Fig. 9 already existed. The progress of this vicious spiral was not rapid, but after the 1990s. The outbreak of shellfish poisoning caused a sharp increase in oysters cultured by the 3-year-culture method because the harvest had to be postponed to the next harvest season, and that caused an acceleration of the spiral. It can be considered that

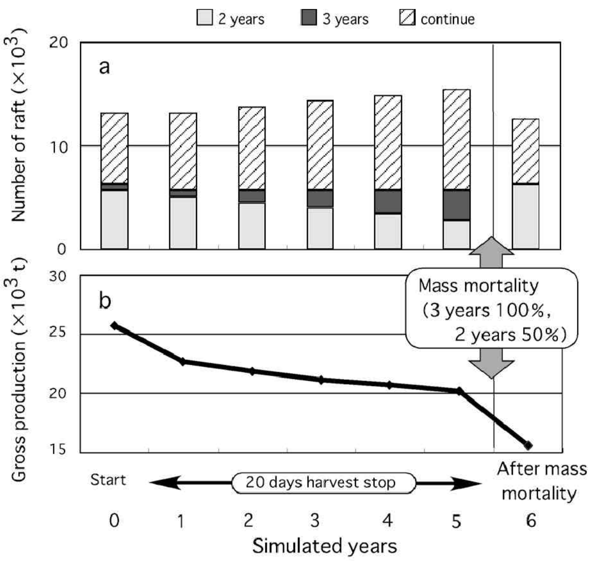

Fig. 7. The simulation of the shortening of the harvest period caused by shellfish poisoning and mass mortality by *H. circularisquama*. a): changes in the number of rafts, and b): changes in gross oyster production in that year.

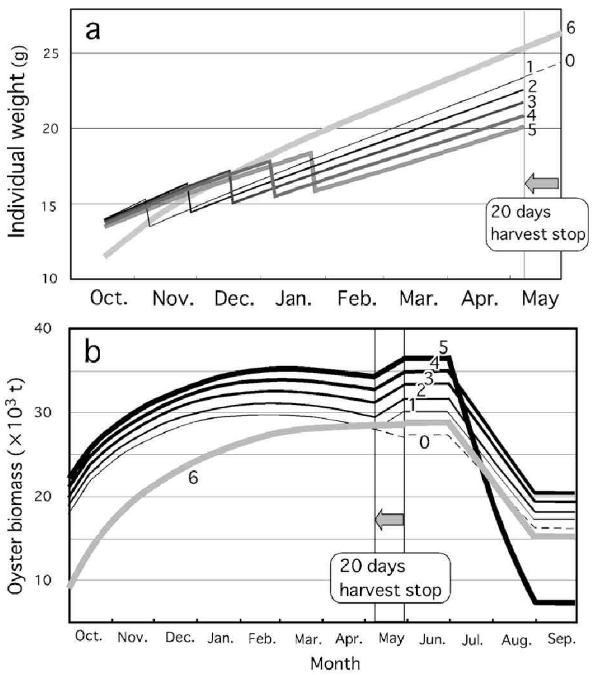

Fig. 8. Changes in the estimated: a) individual weight of oysters, b) oyster biomass under shortening of harvest period caused by shellfish poisoning and mass mortality by *H. circularisquama*. The numbers in this figure correspond to each year in Fig. 7.

dense cultivated grounds, where there is no competition with other plankton and the flow of water is stagnant are favorable environment for *H. circularisquama* to increase (Fig. 9).

Oyster biomass in culture grounds has to be decreased in order to halt this vicious spiral. The most effective method for this purpose is to shorten a culture period. In other words, it would be optimal to culture oyster only by the 2-year culture method rather than combinate with the 3-year one. To confirm this efficacy, we are currently analyzing the carbon flow in an individual scale and in a raft scale. We also plan to combine our analyses, and build an oyster culture model of a culture ground scale in the future.

Acknowledgements

We are grateful to Dr. Paul Kilho Park and Dr. Tetsuo Seki for their comments and suggestion on the manuscript.

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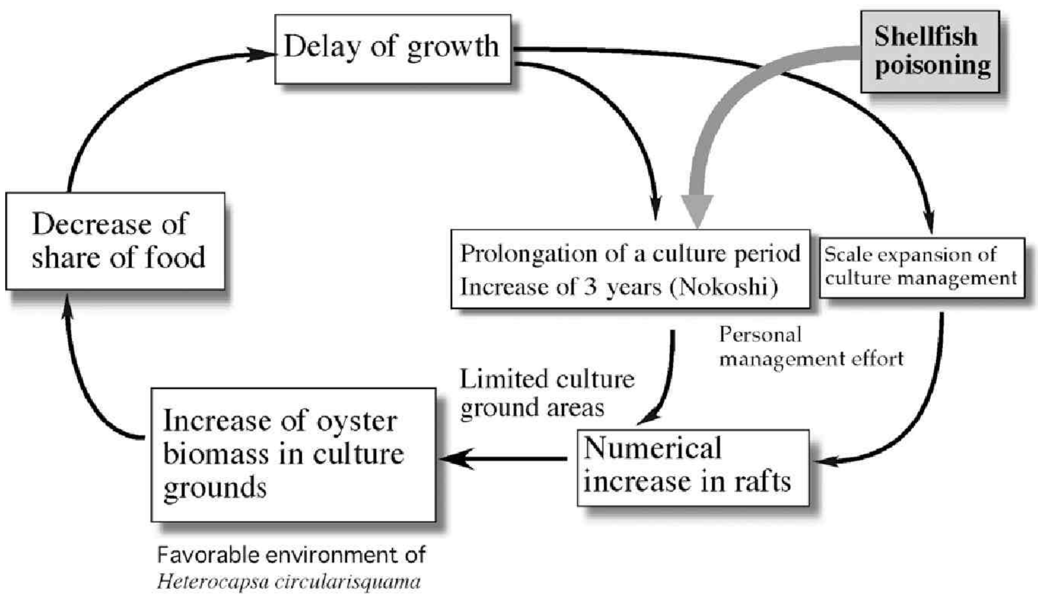

Fig. 9. The vicious spiral of oyster culture in Hiroshima Bay

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水研センター研報，別冊第１号，13－18，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 13-18, 2004

The decline of Manila clam stock in Tokyo Bay

Mitsuharu TOBA*[1]

Abstract The fisheries production of Manila clam in Chiba Prefecture, which amounts to more than 90 % of the total clam production in Tokyo Bay, markedly declined to below 20,000 metric tons (mt) in 1979, after reaching 70,000 mt at its maximum in the late 1960s. This is mainly because of the large-scale reclamation of the shallow tidal areas where clam fisheries were intensively operated. However, the clam production keeps gradual decreasing even after the cessation of the series of reclamation in 1979. The periodical monitoring of clam stocking density shows decreasing of clam production since 1985 seems to be associated with the poor occurrence of wild juvenile clam. In Kisarazu Area, which is the largest Manila clam producer in Tokyo Bay, production is stably maintained between 5,000 and 6,000 mt since late 1980s, in spite of the stocking density of juvenile clam (4-11 mm in shell length) declining from 68 to 12 inds/m^2 during this period. It is probably due to 2,000-3,000 mt of the transplantation of the seed clam (>20 mm) from other area in Tokyo Bay and other prefecture. On the contrary, in Northern Chiba Area, where the clam fisheries relies only upon wild clam stocks without any transplantation, the clam production has sharply declined from around 10,000 mt in late 1970s to 800 mt in 1999 reflecting directly the poor occurrence of wild juvenile clam. The cause of the substantial decline of the wild juvenile clam is still not known.

Key words: Manila clam, Tokyo Bay, stock decline, early life cycle, coastal development

Along the coast of Tokyo Bay, the clam fisheries and culture have been operating in the tidal and shallow water area since before the World War II. Before 1960s, harvested species were mainly hard clam, Manila clam, surf clam, blood cockle, Pacific oyster, and others. However, at the present, only Manila clam and surf clam dominate the bivalve fisheries production.

The annual production of Manila clam in Chiba Prefecture, which occupies most part of the production in Tokyo Bay, reached its peak in late 1960s over 70,000 mt (Fig. 1)*[2]. Then the production declined markedly to below 20,000 mt mainly because of large-scale reclamation of coastal area, where the clam fisheries intensively operated. However, the clam production is only about 8,000 mt in 1999 as a result of continuous and gradual decline even after the cessation of the coastal development. This recent decline in Manila clam production is the major problem of the clam fisheries in Tokyo Bay; however, the exact mechanism responsible for the decline is still not well understood. In this manuscript, I show the details of this problem and several probable factors responsible for this decline.

2003年６月12日受理（Received on June 12, 2003)
*[1] Futtsu Laboratory, Chiba Prefectural Fisheries Research Center, Kokubo 3091, Futtsu, Chiba 293-0042, Japan. 0439-65-3071, E-mail: cbfb@tokyo-bay.ne.jp.
*[2] Statistics of fisheries and culture production of manila clam are quoted from Annual Statistic Report of Agriculture, Forestry and Fisheries in Chiba.

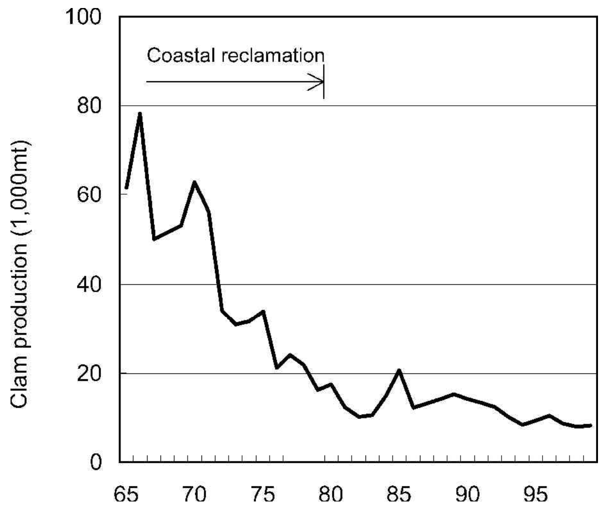

Fig. 1. Annual production of Manila clam in Chiba Prefecture after 1965.

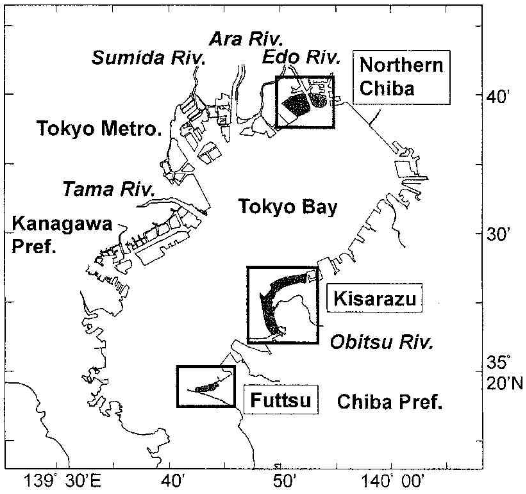

Fig. 2. Culture and fisheries area of Mania clam in Tokyo Bay.

Culture and fisheries areas in Tokyo Bay

Three major areas of clam culture and fisheries in Tokyo Bay, Northern Chiba, Kisarazu, and Futtsu, are located east coast of the bay in Chiba Prefecture (Fig. 2). Each of them has somewhat different operating patterns from the other. For instance, the production in Kisarazu is mainly based on the transplanted clam from outside of the area, while in Northern Chiba production relies only upon its domestic clams that naturally occur. This is due to their historical customs of fisheries as mentioned later, probably being formed through decades under their natural environment and reproductive characteristics of the clam.

Recent variations of Manila clam production in these two areas show quite different patterns; drastic declining in Northern Chiba and relative stability in Kisarazu.

Northern Chiba

Northern Chiba is located in the bottom inside of Tokyo Bay. West, east and North of the area are surrounded by reclaimed land, and Ichikawa Sea Route divides the area into west and east. The bottom level of this area is almost 0 to −2 m in tidal level; the bottom surface is not completely exposed to air even while ebb tide.

As the production in this area relies only upon naturally occurring clams, the clam production has relatively large fluctuation (Fig. 3). With fluctuation, the clam production tends to decline in these years, especially in 1990s. The production in 1999 is only about 800 mt.

Despite recent poor production, fishermen do not transplant any seed clams except during small-scale experimental culture trials. This is because that the naturally occurring clams were so abundant in this area that they did not have to use any stock enhancement for a long time. And also because, this may be the pri-

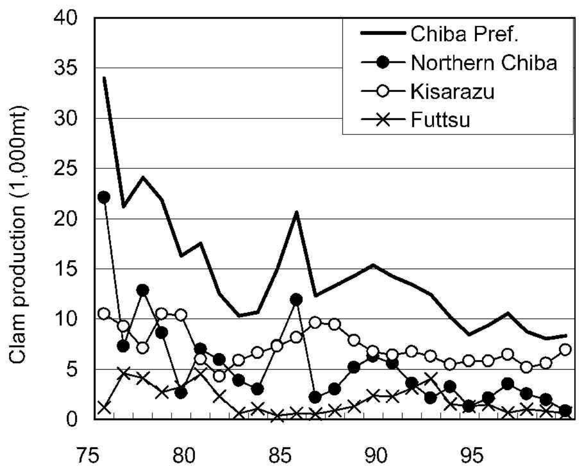

Fig. 3. Annual local production of Manila clam in Chiba

mary reason, the transplantation is highly risky for them economically, as the frequent mortality will collapse the plans of the clam growing and harvesting in this area.

In this area, unpredictable accidental mortality often have occurred. Some of them were caused by Aoshio (blue tide), upwelling of the oxygen-deficient seawater from the bottom of the subtidal zone. When the strong wind blows in the direction from the land to off shore during spring and fall, bottom seawater comes up as the counterbalance of the surface seawater that moves away to offshore (Kakino *et al.*, 1987).

Ichikawa Sea Route, which runs through the middle of the area, is connecting to Edogawa Flood Control Channel. When the water level of Edogawa River rises beyond a certain level due to the heavy rain, such as the case in the typhoon, the floodgate is opened and a great deal of muddy fresh water flows out. Then the salinity of seawater reduces extremely for days, and the mud deposits more than 10 cm in thickness in certain cases and covers the bottom surface for weeks or months. During 15 years from 1985 to 1999, any mass mortality was absent only 6 years in this area (Kakino, 2000). In addition to the mortality of the adult clam by smothering and burying, the mud deposition may inhibit the new recruitment of clam larvae.

Furthermore, population density sharply declines in winter every year (Fig. 4). In 1994-1998, the clam density reached annual peak in summer season, more than hundreds ofinds/m^2, and decreased to below 100 inds/m^2 in winter. Winter mortality is considered to be caused by complex interaction among several factors (Chiba Pref. Fish. Exp. Stn. and Chiba Pref. Fed. Fish. Co-op. Union, 1998). Firstly, the reduction of clam viability by the low temperature and low food condition in winter. Secondly, disturbance of the bottom surface by the stronger wave action in this part of the season. This is related to the subsidence of the land level due to drawing up large volume of ground water during coastal development. By the reduction of the land level, large wave directly reaches deep inside the shallow area. Besides these factors, the predation by some kinds of mallard, which migrate to this area during winter, is also an important factor (Chiba Pref. Engineering Dept. and Chiba Pref. Enterprise Dept., 1999).

Until mid 1990s, the population density of the total clam was constantly above 400 inds/m^2 in spite of the mortalities. That was due to the quick recovery of clam stock after the mass mortality. However, the clam density began to decline rapidly after late 1990s, and it still continues. In particular, the low density of juvenile clam after 1990s is characteristic feature of this area (Fig. 5).

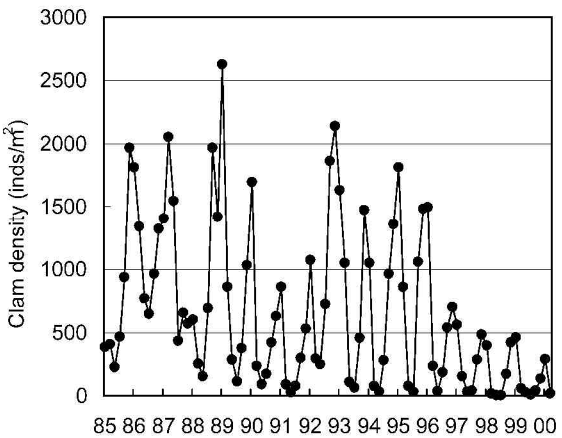

Fig. 4. Seasonal change of the stock density of Manila clam in Northern Chiba.

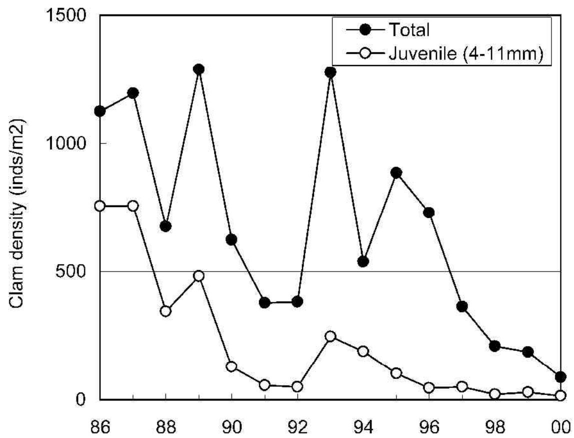

Fig. 5. Changes in the stock density of Manila clam in Northern Chiba.

Kisarazu

The culture and fisheries ground in Kisarazu, which is located in the east coast of the central Tokyo Bay, is the tidal area of bottom level −2 to +1 m in height. The clam production in this area is the largest in Tokyo Bay. The major production of clam in this area relies upon transplanted seed clams. As the density of naturally occurring juvenile clams is relatively low, there remains large tidal area that is used for culture.

The clam production from the transplanted plot from 1986 to 2000 is about 4,000 to 6,000 mt and that from naturally occurring plot is 200 to 1,100 mt. Thus, production from naturally occurring plot is only 6 to 25 % of that of transplanted plot. After 1986, the annual amount of transplanted seed clam, which is introduced from other clam-producing district in southwestern Japan, is 2,200 to 5,000 mt. The shell size of transplanted clam was about 20 mm in length. Recently, the clam size of 25 to 30 mm, which is the minimum size of the clams circulating the commercial market for the human consumption, is used as the seed. This reflects the decline of naturally occurring juvenile clams all over Japan.

The density of naturally occurring juvenile clams in Kisarazu decreased from about 70 to 10inds/m^2 during 1989 and 2000 (Fig. 6). Despite that, the stable total clam production in this area is supposed to be owing to large amount of the seed transplantation. The period of consistent decline of juvenile clam is common to that of Northern Chiba.

Factors common to Northern Chiba and Kisarazu

I think I can indicate some of the probable factors responsible for the decline of clam stock as local and common factors (Table 1). As mentioned above, the poor occurrence of wild juvenile clam is common characteristics to these two areas. Therefore, I am paying attention to the early stage of the life cycle of the Manila clam in Tokyo Bay.

Table 1. Probable factors responsible for the decline of clam

1 Local factors (Northern Chiba) ・Accidental mortality by blue tide (upwelling of oxygendeficient seawater) ・Accidental mortality by river flood ・Winter mortality
2 Common factors to the areas ・Reduction of entire habitat by the coastal reclamation ・Contraction of the effective spawning period ・Larval mortality due to oxygen deficient seawater

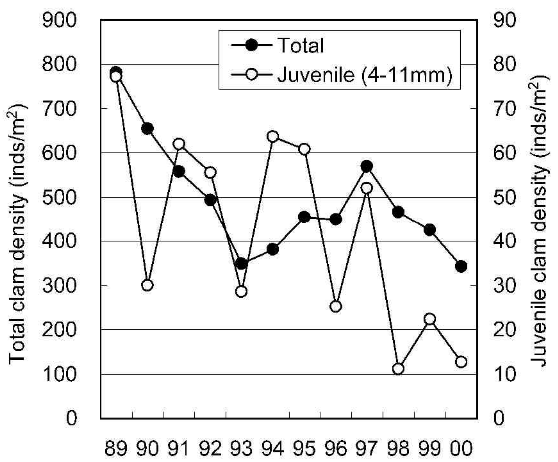

Fig. 6. Changes in the stock density of Manila clam in Kisarazu.

One of the most important factors influencing the decline of clam stock may be reduction of the habitat area (Fig. 7). Results of the investigation in 1945 and 1985 show that nearly 90 % of the tidal area was lost.

Manila clam has the spawning period in warm water season; May to October in Tokyo Bay. Newly hatched larvae have a strong negative geotaxis, and 2 to 4 weeks of swimming period until settlement. From the results of the studies on the seawater current, the larvae, which are spawned at the coastal area, may be dispersed and transported throughout the bay, and mixed during the swimming period. The bay area may play an important role as the habitat of the larval stage for the Manila clam

population in Tokyo Bay. While the shallow or tidal coastal area has reduced greatly in the past few decades, the bay area is almost constant. The reproductive potentiality of the Manila clam in Tokyo Bay is reduced by decline of the total fecundity of the adult clam. Furthermore, the larvae dispersed all over the bay have much less chance to get back to the scattered shallow areas where they can settle.
Manila clam has two peak periods of gonadal maturation in a year; spring and autumn in Northern Chiba. While the fecundity of clam seems greater in spring spawning (Toba *et al.*, 1993), adult density in spring is very low because of the winter mortality. The clam population in Northern Chiba may not spawn effectively in spring season.

The peak spawning period of Manila clam in Kisarazu is suspected to be once in a year during summer months from the observation of the seasonal changes in condition factor. And the transplanted clams that have different seasonal reproductive patterns from native clams may influence the low peak values of condition factor in the clam population in Kisarazu.

Three peaks of the larval density were recorded in post summer months, August, September, and November at the adjacent points of Northern Chiba from May to December in 2001. The peaks of postlarval density, smaller than 0.4 mm in shell length, were observed 2 to 4 weeks after the peaks of larval abundance. In Kisarazu as well, the peaks of the larval and postlarval density were observed in post summer months during 2000 and 2001. Substantial spawning and postlarval recruitment for the clam population may contract to post summer in Tokyo Bay in these years.

During the warm water season from May to October, the bottom layer of the large part in bay area is covered by oxygen-deficient seawater (Ishii, 2003). The thickness of oxygen-deficient layer often grows more than several meters especially in north bottom side of the bay. Although the thickness of oxygen-deficient bottom layer gets lowered temporally by the stormy weather, it builds up quickly again under the calm weather within few days. Thus, oxygen-deficient seawater lies intermittently during whole warm water season. As the larval swimming activity is markedly reduced in oxygen-deficient seawater, continual appearance of oxygen-deficient layer may affect the survival of larval population in Tokyo Bay for months.

Conclusion

I suspect that not only the sharp decline of Manila clam production before late 1970s in

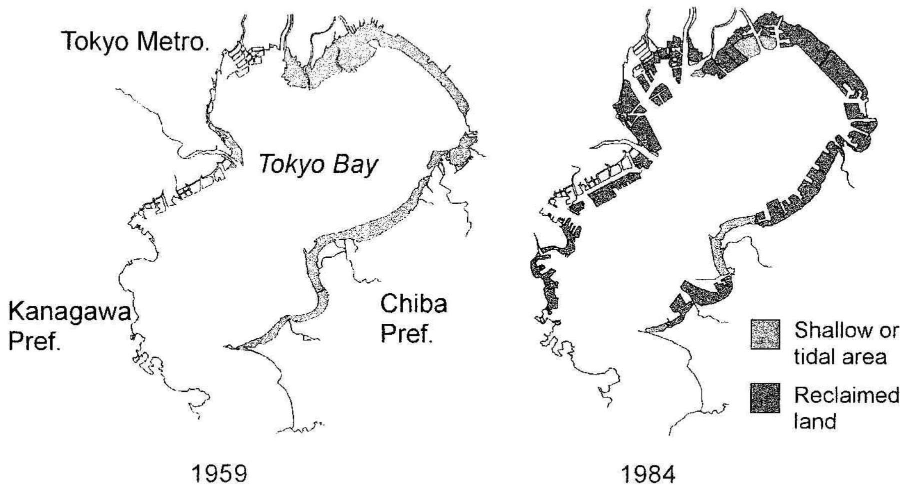

Fig. 7. Reduction of the clam habitat in Tokyo Bay during 1959-1984.

Tokyo Bay but also the gradual decline afterwards may be affected by the series of the coastal reclamation that ceased in 1979. One of the marked and common characteristics of the clam populations in local areas during latter period is continual decline of the recruitment of natural juvenile clams. Reduction of adult population, contraction of reproductive period, larval mortality caused by oxygen-deficient seawater, and reduction of shallow or tidal area for larval settlement may involve poor recruitment of juvenile clam. Temporal mass mortality including adult population caused by the blue tide, river flood, low temperature and wave action, predation by the birds seems to accelerate the decline of clam stock.

The ecological impact on the clam population by the artificial modification of their habitat and hypertrophication of the bay area may appeare through certain period as long as more than 20 years. Unfortunately, however, as mentioned before, the exact mechanism responsible for the decline is still not well understood.

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水研センター研報，別冊第１号，19－31，平成16年
Bull. Fish. Res. Agen. supplement No. 1, 19-31, 2004

Environmental conditions relevant to aggregative distribution of macrobenthos below coho salmon culture cage

Ryo SASAKI* and Akio OSHINO*

Abstract Actual changes in environmental conditions relevant to aggregative distribution of the macrobenthos below coho salmon culture cage were examined by diving observation at Onagawa Bay in 1990s. Organic sediment derived from leftovers of moisture food pellets and fish feces were 15 cm in height at the center below culture cage. Dominant species of macrobenthos were identified *Nebalia bipes*, *Schistomeringos japonica*, *Melita* sp. and *Capitella spp*. Highest density of *Nebalia bipes* was found ca. 40,000 inds./m^2 and that of *Schistomeringos japonica* was ca. 5,000 inds./m^2 from enriched sediment on the bottom surface. Aggregative distribution of *Nebalia bipes* and *Schistomeringos japonica* were monitored at 10-m distance zone from the center point in summer, and that of *Nebalia bipes* and *Melita* sp. were monitored within 5-m distance from the center in winter. From the ecological viewpoint for these external distributions, aggregative position of macrobenthos was correlated to the marginal zone of enriched sediment. Biological activities so-called bioturbation were recognized in conjunction with synchronous patterns of the distribution between macrobenthos and organic sediment below culture cage.

Key words: coho salmon, macrobenthos, environmental condition

The work described here has the aim of obtaining information on the ecological relationship between decomposition of organic sediment and distribution of macrobenthos below coho salmon culture cage. To assess the impacts of fish farming and to prevent self-induced deterioration, several criteria for the protection of the environment around aquaculture farms have been proposed and summarized (Yokoyama, 2000). In order to establish the most prudential plan to prevent environmental deterioration around fish farm site, we need to understand the following ecological basis. Firstly, promotion of circulated nutrient linkage converted by mineralization from the loaded organic sediment decomposed through bacteria and macrobenthos toward ongrowing of sedentary fish, which is regarded as a part role of the cyclic processes in natural ecosystem. Secondary, retrieval of economical materials such as a sedentary fish which converted by macrobenthos with enriched sediment in the course of fishing is a removal step for discharging the organic loads to outside of ecosystem through food chain in the benthic community (Tamai, 1990).

Ohmori *et al.* (1995) has already estimated on the organic loads derived from coho salmon culture by analyzing culture records of fisherman's annual diary, and pointed out that low oxygen levels were yearly correspondent to the increasing production of coho salmon at Onagawa Bay during early 1990s. Key problem to study actual changes in environmental

2003年６月12日受理 (Received on June 12, 2003)
* Kesennuma Miyagi Prefectural Fisheries Experimental Station, 119 Hajikami, Kesennuma, Miyagi 988-0247 Japan.
Email: Kssuisi@pref.miyagi.jp

conditions below fish culture cage lies in improving the methods for monitoring organic sediment and macrobenthos, which sample was indirectly obtained with Ekman-Birge grab conducted by surrounding researcher on the boat. This lack of understanding is mainly due to underwater research activity and also due to difficulty in sampling methods for macrobenthos below culture cage where environmental conditions are dark, dirty and dangerous for research diver.

In addition to the finding on the nitrogen loads to the environmental water caused by the feeding (Ohmori *et al.*, 1995), we tried to find out the correlationship between the aggregative distribution of macrobenthos and loaded organic sediment that needed to establish the actual contribution for preventing the deterioration. Furthermore, effects of shell-collector as a habitat for macrobenthos were investigated to promote the decomposition of organic sediment and artificial improvement for a bio-monitoring function of environmental condition on the bottom surface below culture cage.

Materials and Methods

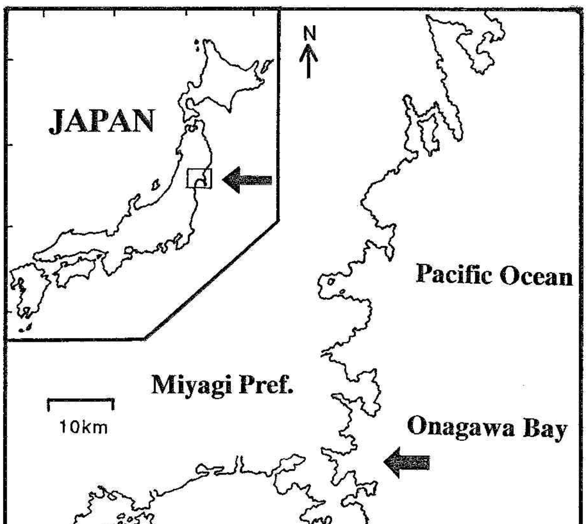

Fig. 1. Location of northern part of Japan and coast map of Miyagi Prefecture showing the study site

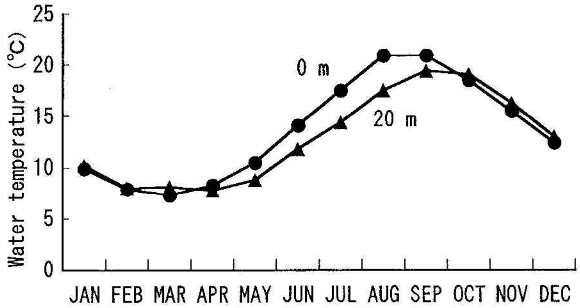

Fig. 2. Monthly changes in water temperature of surface and 20-m depth layer in Onagawa bay

Onagawa Bay (38° 24'N, 141° 29'E), which has an area of 12 km^2 and a mean depth of 19 m, is a typical embayment where mariculture farms, e.g. oyster, scallop, and coho salmon, are densely distributed (Fig. 1) . Water temperature of surface ranged from 21 ℃ in August and to 7 ℃ in February, and that of 20-m depth ranged from 19 ℃ in September to 8 ℃ in March, respectively. Depth of the study site was 20 m and bottom water temperature between August and October during post harvest time were more than 18 ℃ (Fig. 2).

Coho salmon culture is generally started from November with 150 g of body weight for a juvenile and continued until following July with 3 kg body weight for a harvest size, so that absent periods for culture attains 3 months from August through October. Initial density of juveniles in a cage was approximately 15,000 and harvest time 10,000 individuals. The size of culture cage was a regular square of 13-m long with 10-m depth net.

Comparing the correspondence between trend of coho salmon production and environmental conditions in bay-wide, macroscopic change of dissolved oxygen as a bottom water condition and total sulfide value as a sediment condition were reexamined by the monthly recording data-book on environmental survey of Onagawa Bay (Miyagi Prefecture, 1982〜2000).

Dissolved oxygen at surface and bottom water was measured monthly from June through November 1996 in the vicinity of studying culture cage. Bottom sediments were collected by the core-tube of 6-cm diameter with a cap. Core samples were carefully collected by diver's hands at each 5-m intervals from the center point toward out-skirt with 30-m-long distance by 5-m intervals.

In order to estimate the influence of fish culture, the scale-bar marked 5-cm interval recognizing for the degree of sedimentation was set by diver at the center point of the bottom surface below culture cage in May 1996. Height of organic sediments before and after harvest derived from leftovers of moisture food pellets and fish-feces were recorded by underwater camera once in a month until following years. Monthly fluctuations of total sulfides (TS), chemical oxygen demand (COD) and ignition loss (IL) values of bottom sediments regarded as the organic indicator were monitored to define a boundary of the enriched area at each 5-m intervals from the center point below culture cage.

In addition to the measurement of environmental conditions, spatial and temporal distribution patterns of macrobenthos were compared below culture cage. Bottom sediments in a 0.2 m^2 was dragged for collecting macrobenthos with 1-mm mesh net by diver's hands from the center point toward out-skirt of 30-m-long distance by 5-m intervals in July, September, December, 1996 and February 1997.

In the course of research activities, feeding of moisture food pellets for the culture cage was discontinued at this study site after July 1996 by the cessation of culture operation with cost disadvantage. After then, distribution of macrobenthos and TS, COD and IL value of sediment were traced at the same center area below culture cage until August 1997 to compare with those of the environmental conditions in July 1996 of the harvest time.

In order to examine the dependency of *Nebalia bipes*, a dominant species of macrobenthos in the study site, shell-collector bag as a habitat for shelter substratum was set on the bottom surface at each 5-m intervals from the center point. Shell-collector bag was composed by 10 pieces of oyster shells. The control sample against shell-collector bag was collected at the direct vicinity of each area by the same square.

Besides outdoor observations for the distribution pattern of macrobenthos, feeding responses between sedentary fishes distributed around culture cage against macrobenthos were confirmed by the indoor rearing experiment, which frozen Nebalia bipes and *Schistomeringos japonica* were put into the

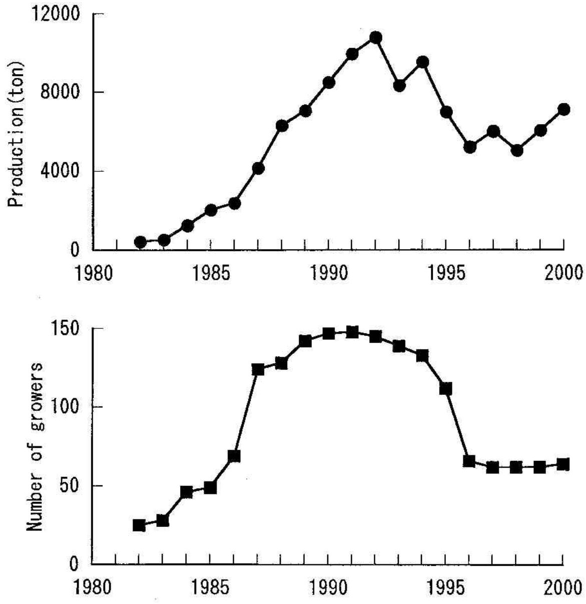

Fig. 3. Trends in production and growers of coho salmon culture in Onagawa bay, Miyagi Prefecture 1982 - 2000

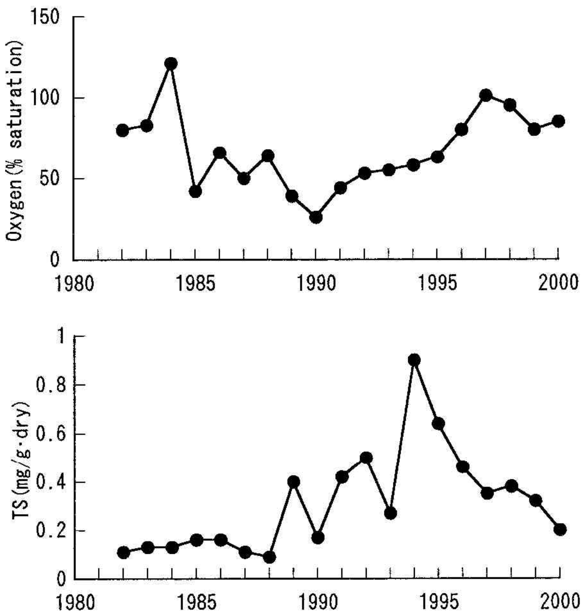

Fig. 4. Yearly changes in dissolved oxygen value of the bottom water in July and total sulfide value of the bottom sediment at August in coho salmon culture ground, 1982-2000

tank fed for several species of fishes.

Results and Discussion

1. Environmental conditions at coho salmon culture area

Production of coho salmon culture in Onagawa Bay has developed increasingly from 1980s, then matured in early 1990s and decreased in subsequent several years including cessation of operations due to cost disadvantage. The total production reached a peak in 1992 when 10,769 ton were landed with 148 growers, and bottomed in 1998 when 5,021 ton were landed with 62 growers (Fig. 3).

Changes in dissolved oxygen saturation as a bottom water condition in summer of early 1980s ranged between 120 % and 80 %, after then oxygen value decreased abruptly and attained lowest in 1990 with 26 %, and recovered yearly until 2000 with 85 % (Fig. 4). Changes in total sulfide value as a sediment condition of coho salmon culture ground also attained a highest value in 1994 with 0.9 mg/g・dry・sedi ment, which tend to follow the deterioration of water condition and recovered yearly until 2000 with 0.2 mg/g・dry・sediment. Both oxygen concentration and total sulfide value were subsequently appeared in corresponding to those peak years by over-production of coho salmon culture. In recent years, the relationship between the production of coho salmon and decreasing value of dissolved oxygen and total sulfide were regarded as a balanced load between organic deposit by culture production and decomposition by natural environment. In relation to this tendency, Yokoyama (1995) described that large biomass at the innermost part of Ohmura Bay suggest that a relatively high concentration of sulfide (0.45-0.75 mg/g・dry sediment) never prevents distributions of animals, and that organic enrichment of the sediments is sufficient to provide a rich food source but is not yet high enough to cause serious oxygen depletion .

Organic loads in bay-wide derived from coho salmon culture with feeding moisture pellets has already estimated at the same area in early 1990s by analyzing culture records of fisherman's diary (Ohmori *et al*, 1995). According to the estimation of each parameter, annual amount of organic loads as equivalent to nitrogen discharge was calculated 6,767 kg a grower from total amount of food supply. This amount of nitrogen divided into 1,640 kg as 25 % for landing of culture production and 5,127 kg as 75 % for discharging to the environmental waters (Fig. 5). Nitrogen load against the environmental waters was corresponded to 9 % of total landing of cultured coho salmon, so that annual nitrogen load was estimated approximately 2 ton/day in Onagawa Bay (Ohmori *et al.*, 1995).

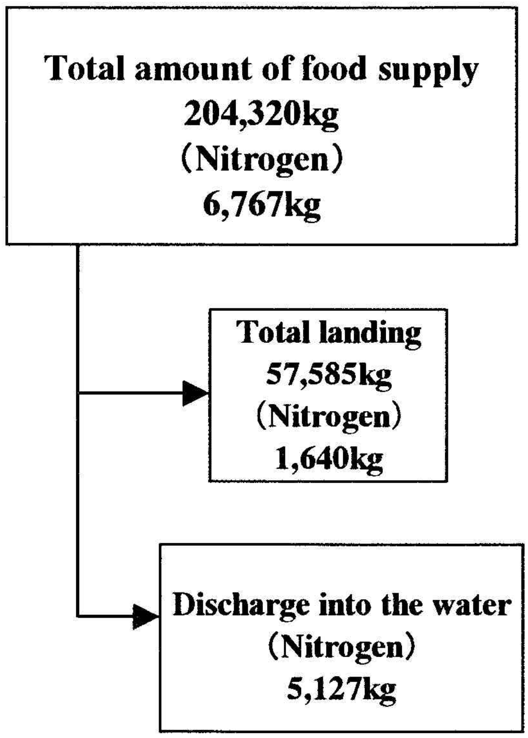

Fig. 5. Annual amount of organic loads and equivalent nitrogen discharge for a grower of coho salmon culture (after Ohmori *et al.* 1995)

2. Environmental conditions below culture cage

Saturation of dissolved oxygen at surface water in the study site was 96 % in average, ranging 137 % at highest in June and 76 % at lowest in October, and that of bottom water was 73 % in average, ranging 53 % at lowest in September and 84 % at highest in November.

Monthly changes in the height of sediment derived from leftovers of moisture food pellets and fish feces was directly measured by scale-bar set below the center of culture cage (Fig. 6). Maximum sedimentation was monitored 15 cm in height at the harvest time in July 1996, and then decreased month by month until following February 1997 when original ground was again exposed through the decomposition processes of organic sediment (Fig. 7). Feeding was ended by culture cessation in July 1996. Therefore, it was evident that the time for decomposition of organic sediment derived from food supply requires nearly 7 months under natural environmental conditions. The volume of organic sediment at harvest time was estimated approximately 37 m^3 below culture cage, which was calculated from the distributed range of sedimentation within 25-m diameter measured by diver.

The TS, COD and IL values of bottom surface were monitored to define a boundary of the enriched sediments collected at each 5-m intervals from the center point toward outer area below culture cage, which expressed the marginal zone decomposed organic sediments by benthic communities (Fig. 8). A peak of TS values was 1.22 mg/g・dry sediment at 10-m distance in July, 1.98 mg/g・dry sediment at 5-m distance in September and 1.65mg/g・dry sediment at 5-m in December 1996. A peak of COD values was 270mg/g・dry sediment at the center in July, 255 mg/g・dry sediment at 5-m distance in September, 182 mg/g・dry sediment at the center in December 1996 and 104 mg/g・dry sediment at the center in February 1997. A peak of IL values was 59 % at the center in July, 42 % at 5-m distance in September, 35 % at the center in December 1996, and 26 % in February 1997.

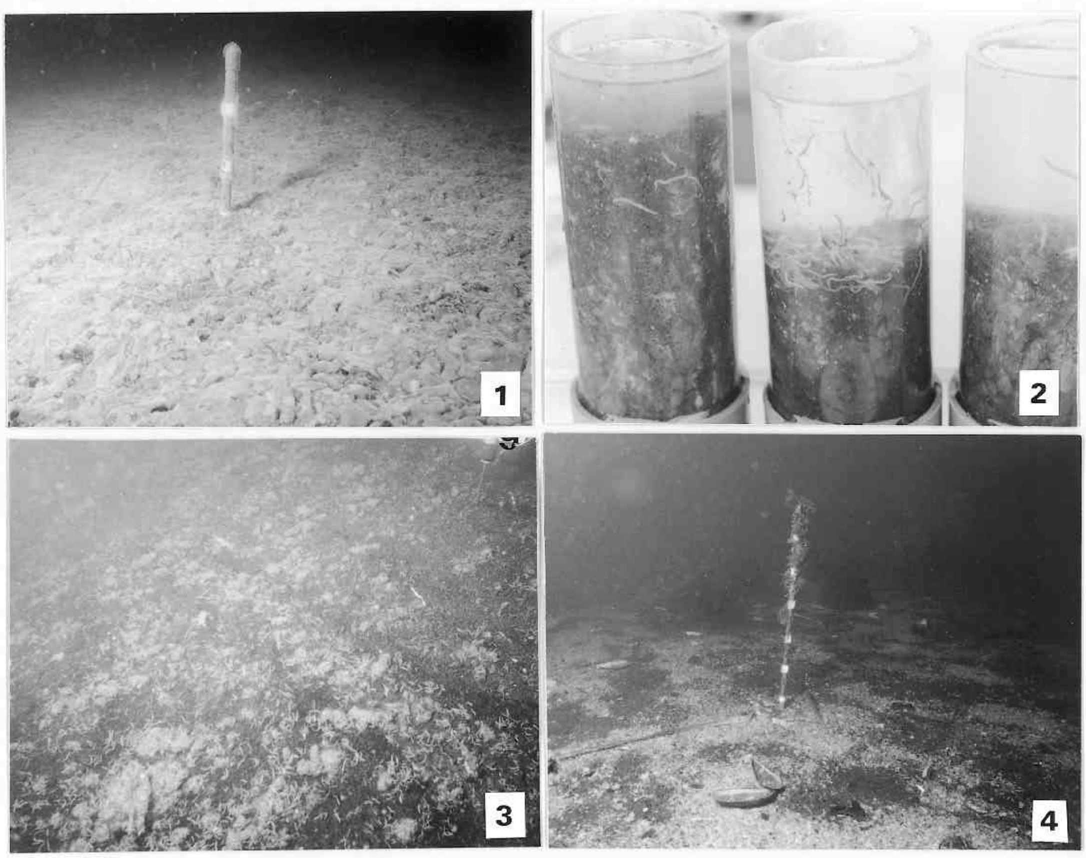

Fig. 6. 1 : Scale-bar in the bottom sediment at harvest time (photographed in July 1996). 2 : Contents of core tube samplers viewing from lateral side. 3 : Aggregative distribution of *Schistomeringos japonica* on the bottom sediment. 4 : Scale-bar in the bottom restored original condition (photographed in Feb. 1997)

According to the change in a peak position of COD and IL values below culture cage, simultaneous fluctuations corresponded to each month indicate removable contraction of organic sediment from outer marginal zone toward the center area through the decomposition processes of organic sediment. The TS, COD and IL values as a chemical indicator of the bottom conditions suggest in general that the influences of organic sediments were limited within 10 to 15-m distance area from the center point in summer and within 5-m distance in winter through the synchronous contraction. In relation to these findings, azoic conditions and high TS value (over 1.3mg/g･dry sediment) were found at the fish farm in Gokasho Bay during the summer, which suggested a key factor in eliminating the macrofauna in organic enriched habitat and significant negative correlation between the sulfide content and the density of the macrofauna (Yokoyama *et al.*, 1997).

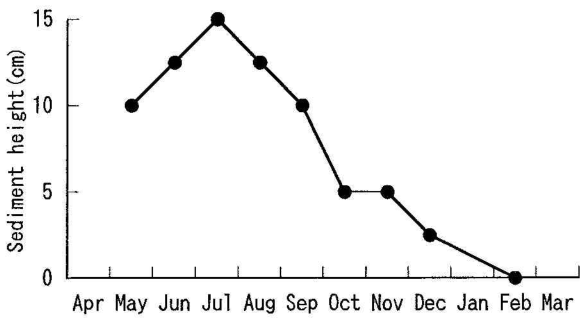

Fig. 7. Monthly changes in the height of sediment derived from leftovers and fish feces below culture cage monitored by scale-bar

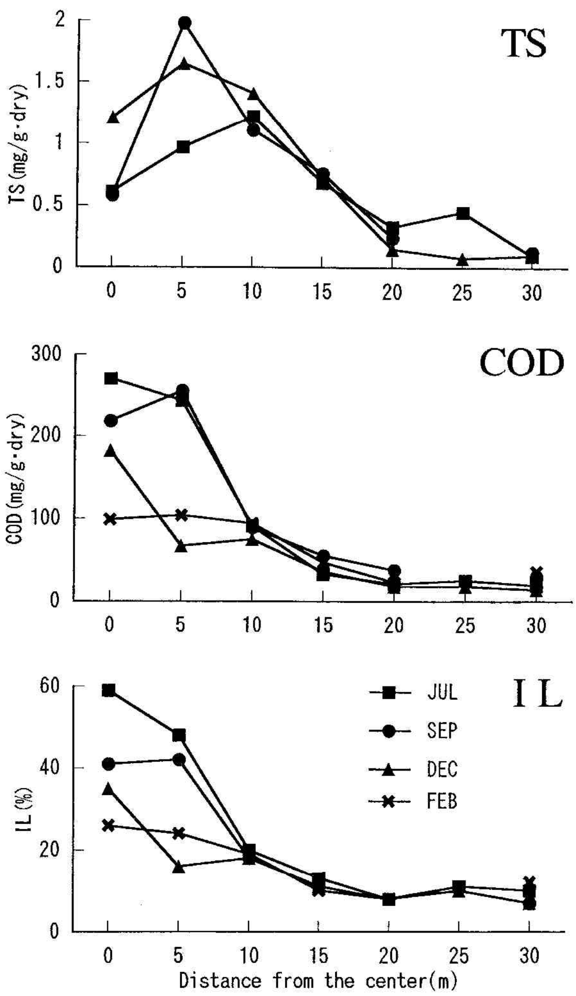

Fig. 8. Changes in total sulfide, chemical oxygen demand and ignition loss values of bottom sediment collected from each 5-m intervals within 30-m distance from the center point below culture cage

3. Distribution of macrobenthos below culture cage

Dominant species of macrobenthos collected by dragging with hands-net through bottom

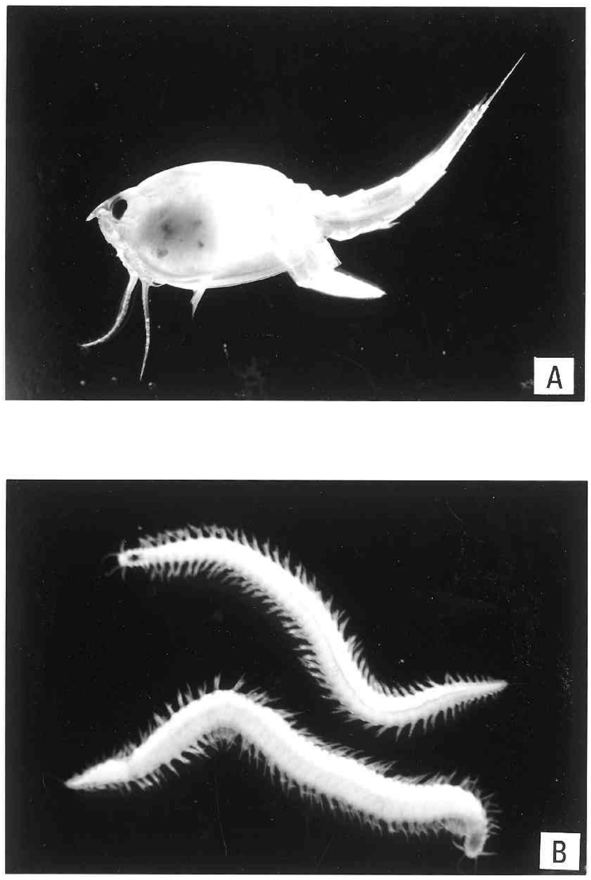

Fig. 9. Photographs of *Nebalia bipes* (A) and *Schistomeringos japonica* (B)

sediment below culture cage were identified as *Nebalia bipes* and *Schistomeringos japonica* (Fig. 9).

From the indoor observations under rearing conditions, the response of *Nebalia bipes* to the light is strongly photonegative, hiding and burrowing underneath bottom sediment in the daytime and swarming up and crawling around bottom surface in the dark. *Nebalia bipes* behaves as carnivorous feeder, which tends to cause economical damage for coastal fishing by aggregating and feeding the fishes left, caught within gill-net (Nishimura and Hamabe, 1964). The habitat of *Nebalia bipes* seems to be widely adopted to low oxygen conditions under chemically reduced environment. Maturing adults holding larvae within brood-pouch were collected in summer and subsequent juveniles were appeared in autumn. Average body length measured between carapace and telson were 6.9 mm in April, 5.7 mm in August, 4.9 mm in November 1996 and 4.2 mm in January 1997. Yokoyama *et al.* (1997) pointed out that *Nebalia bipes* had its maximum density (700 inds./m^2) at fish farm site of Gokasho Bay in May, probably due to the deoxygenation of the bottom water accompanied by increasing temperatures and increasing activities of fish farming.

From the field observations, *Schistomeringos japonica*s aggregates to the leftovers, fish feces and empty shells of fouling animals *etc.*, which distributed on the surface of sediments covered ordinarily by sulfur bacteria *Beggiatoa* spp. The habitat and behavior of *Schistomeringos japonica*s seems to be similar to that of *Nebalia bipes*, which originally adopted to chemically reduced environment. Maturing adults of *Schistomeringos japonica*s were recognized by its visible gonad through the translucent skin with body length ca. 10 mm in summer, and subsequent juveniles were appeared in autumn. *Schistomeringos japonicas* adapted for a tolerance to azoic conditions and distributed frequently in the byssus of *Mytilid* communities under polluted waters. It is supposed that this species performs important role for decomposition of organic sediments by grazing the substratum with its developed jaws under azoic conditions (Miura, personal communication).

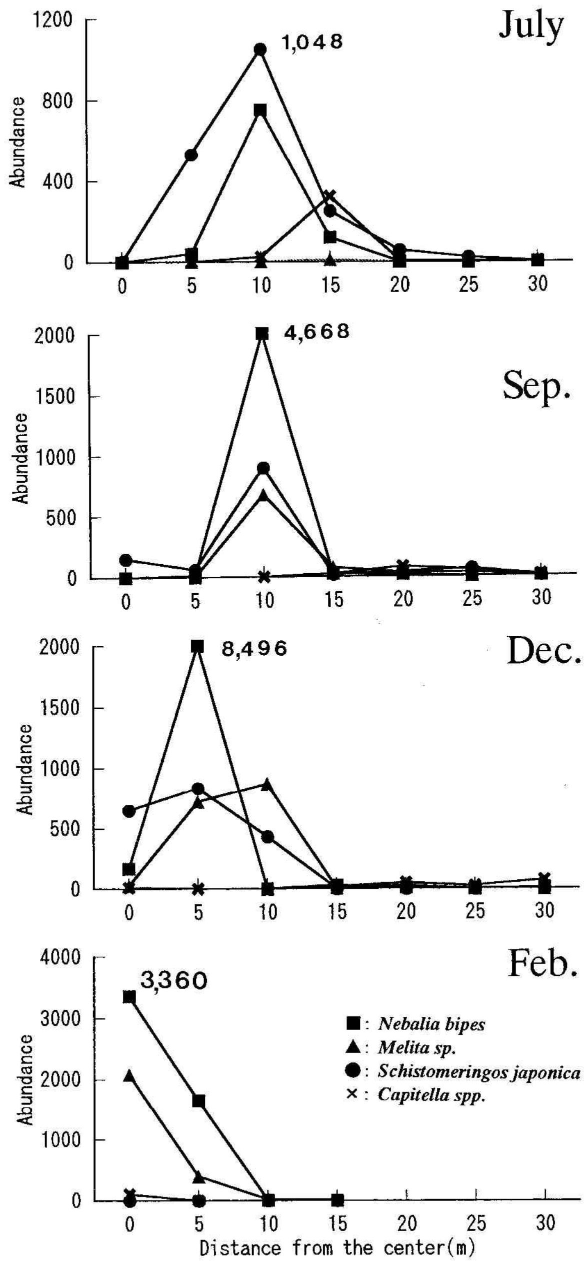

Fig. 10. Changes in the aggregative distribution of the macrobenthos (■: *Nebalia bipes*, ▲: *Melita* sp., ●: *Schistomeringos japonica* and ×: *Capitella* spp.) collected in 0.2 m^2 from each 5-m intervals within 30-m distance from the center point below culture cage

Seasonal changes in the aggregative distribution of dominant macrobenthos collected by 0.2-m^2 sediment at each 5-m intervals within 30-m distance from the center point below culture cage were shown in Fig. 10.

In the distribution of macrobenthos collected In July, highest density of *Schistomeringos japonica* was found 5,240 inds./m^2 at 10-m distance from the center, that of *Nebalia bipes* was found 3,760 inds./m^2 at 10-m distance and that of *Capitella* spp. was found 1,600 inds./m^2 at 15-m distance. *Schistomeringos japonica* and *Nebalia bipes* dominated from 5-m to 15-m with a peak at 10-m distance from the center point. Fouling organisms including oyster, mussel, barnacle etc. fell down to the bottom from culture facilities, which materials exposed on the bottom surface seem to be suitable habitats as shelters for *Schistomeringos japonica* and *Nebalia bipes*.

Distribution of macrobenthos collected in September, highest density of *Nebalia bipes* was found 23,340 inds./m^2 at 10-m distance from the center, that of *Schistomeringos japonica* was found 4,500 inds./m^2 at 10-m distance, that of Melita sp. was found 3,360 inds./m^2 at 10-m distance and that of *Capitella* spp. was found 400 inds./m^2 at 20-m distance. *Nebalia bipes*, *Schistomeringos japonica* and *Melita* sp. were dominant in order at 10-m distance from the center point. Comparing with the density of July, the number of matured individuals of *Schistomeringos japonica* was decreased by assuming mortality due to spawning. It was evident that those macrobenthos tend to aggregate around the immediate vicinity besides organic sediments and impoverish in a center area where organic sediments were abundant.

In the distribution of macrobenthos collected in December, highest density of *Nebalia bipes* was found 42,480 inds./m^2 at 5-m distance from the center, that of *Schistomeringos japonica* was found 4,160 inds./m^2 at 5-m distance, that of *Melita* sp. was found 4,350 inds./m^2 at 10-m distance and that of *Capitella* spp. was found 240 inds./m^2 at 20-m distance. In December, distribution pattern of macrobenthos were synchronously removed toward the center from out-skirt area and concentrically limited within 10-m distance around the center point. Matured individuals of *Schistomeringos japonica* were already disappeared and only juveniles dominated within 10-m distance zone. High abundance of juveniles of *Nebalia bipes* was characteristically found within 5-m distance zone from the center point. Fouling empty shells and particle fish-bones derived from raw fish in moisture pellets were exposed again on the natural ground, which corresponded to the decomposition of organic sediment below culture cage.

Distribution of macrobenthos collected in February, highest density of *Nebalia bipes* was found 16,800 inds./m^2 at the center, that of *Melita* sp. was found 10,400 inds./m^2 at the center and that of *Capitella* spp. was found 520 inds./m^2 at the center. In February, distribution pattern of *Nebalia bipes* and *Melita* sp. were concentrated within 5-m distance from the center point, which organic sediment enriched macrobenthos for the nutrient consumption as a terminal area below culture cage.

Considering the distribution pattern in respect of each species, highest density of *Nebalia bipes* was 42,480 inds./m^2 at 5-m distance from the center in December 1996, that of *Melita* sp. was 10,400 inds./m^2 at the center in February 1997, that of *Schistomeringos japonica* was 5,240 inds./m^2 at 10-m distance in July and that of *Capitella* spp. was 1,600 inds./m^2 at-15m distance in July, respectively .

According to synchronous patterns of the distribution between macrobenthos and organic sediment properties which was concerning the environmental viewpoint with the chemical indicator by TS, COD and IL values at each season, aggregating sites of each macrobenthos were closely correlated to the marginal zone of enriched sediment below culture cage.

At the time of 1-year discontinuance after culture cessation in July 1996, chemical indices of organic sediments collected at the center area in July 1997 were changed from 0.79 mg/g・dry sediment to 0.65 mg/g・dry sediment with TS value, from 257 mg/g・dry sediment to 151 mg/ mg/g・dry sediment with COD value and from 54 % to 26 % with IL value, respectively. Highest density of macrobenthos at the same area was changed from 3,760 inds./m^2 to 110 inds./m^2 with *Nebalia bipes*, from 5,240 inds./m^2 to 260 inds./m^2 with *Schistomeringos japonica*, from 50 inds./m^2 to 215 inds./m^2 with *Melita* sp. and from 1,600 inds./m^2 to 5,125 inds./m^2 with *Capitella* spp. Comparing with the data between 1996 and 1997, it was supposed that the habitat evaluation below culture cage of *Nebalia bipes* and *Schistomeringos* was decreased and that of *Melita* sp. and *Capitella* spp. was increased in accordance with those nutrient conditions indicated chemical indices mentioned above.

From the indoor rearing observation by feeding response of *Hexagrammos otakii* (greenling), *Sebastes inermis* (black rockfish), *Limanda yokohamae* (marbled sole) and *Kareius bicoloratus* (stone flounder), *Nebalia bipes* and *Schistomeringos japonica* were almost fed with a desirable reaction. Comparing the reaction time for feeding by these sedentary fishes between *Nebalia bipes* and *Schistomeringos japonica*, those fishes tended to prefer *Nebalia bipes*. It was suggested that *Nebalia bipes* and *Schistomeringos japonica* were treated for suitable bait against adjacent sedentary fishes distributed around the enriched sediment below culture cage. Furthermore, dispersion by ongrowing and retrieval by fishing of these sedentary fishes are regarded as a removal step for macrobenthos propagated by enriched sediment, which has a responsibility for promoting discharge of organic loads to outside of ecosystem through food chain in the benthic community at the fish farm site.

4. Evaluation of shell-collector

Considering the distribution of macrobenthos below culture cage, *Nebalia bipes* dominated in a year-around and recognized as effective species for decomposition of loaded organic sediment. Spatial and temporal distribution patterns of *Nebalia bipes* were re-examined by the comparative observation conducted within and without shell-collector set

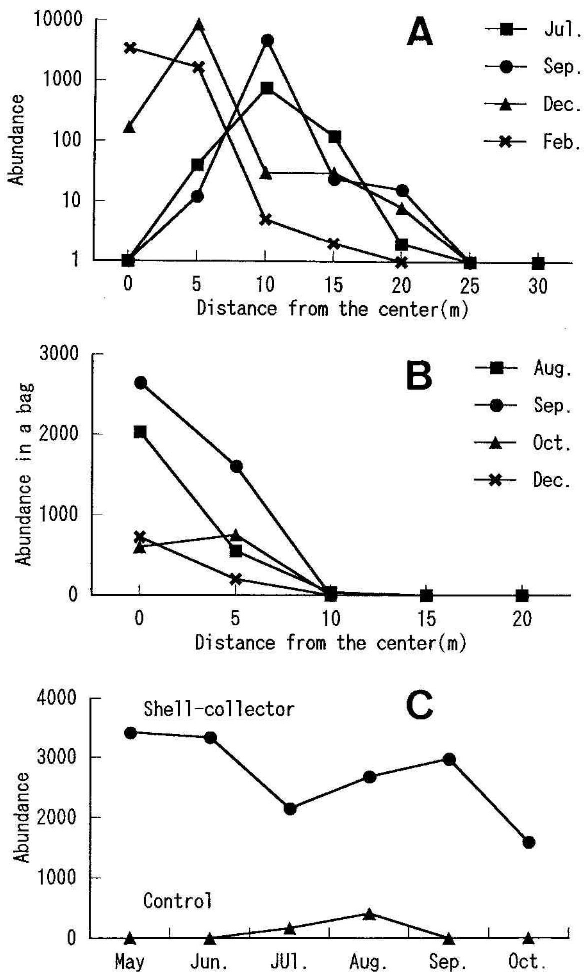

Fig. 11. Spatial and temporal distribution of *Nebalia bipes* below coho salmon culture cage. A: seasonal changes in the density in 0.2 m^2 and aggregative position on bottom surface , B: seasonal changes in the aggregative position distributed within shell-collector bag, C: comparison of monthly density between shell-collector bag and bottom surface in 0.2 m^2

for a monitoring of shelter substratum (Fig. 11). Distribution of *Nebalia bipes* was delineated with aggregative pattern in a certain position, which location was seasonally shifting from outer area toward the center point corresponded to the marginal zone of enriched sediment under natural conditions (Fig. 11A). The reason for shifting distribution was supposed that the behavior of *Nebalia bipes* depended highly on the habitat with shelter materials including empty shells and so on that were exposed subsequently on the bottom surface by sediment decomposition with passing of the season.

The average number of *Nebalia bipes* distributed within shell-collector in summer (August and September) attained 2,335 individuals at the center and 1,075 individuals at 5-m distance zone. Furthermore, that of *Nebalia bipes* distributed within shell-collector in autumn (October and December) attained 590 individuals at the center and 817 individuals at 5-m distance zone, respectively (Fig. 11B). It was evident by the indoor rearing observation that *Nebalia bipes* expressed a photonegative response and a high dependency with substratum such as shell-collectors like a shelter for habitat in daytime. On the other hand, the number of *Nebalia bipes* without shell-collector distributed in natural bottom condition was almost nil at the center area during summer and autumn, the aggregative distribution of *Nebalia bipes* was limited outer area than 5-m distance zone without in winter as shown in Fig. 11A.

On the monthly changes in the density of *Nebalia bipes* from May through October 1996 collected at the center area below culture cage, the average number of *Nebalia bipes* distributed within shell-collector was 2,684 individuals and that without shell-collector was 97 individuals, respectively, density variance of 28 times (Fig. 11C). Comparing the efficiency of shell-collector between oyster and scallop shells, the average number of *Nebalia bipes* was 2,940 individuals with oyster and was 2,330 individuals with scallop. On the other hand, that of control sample collected direct vicinity

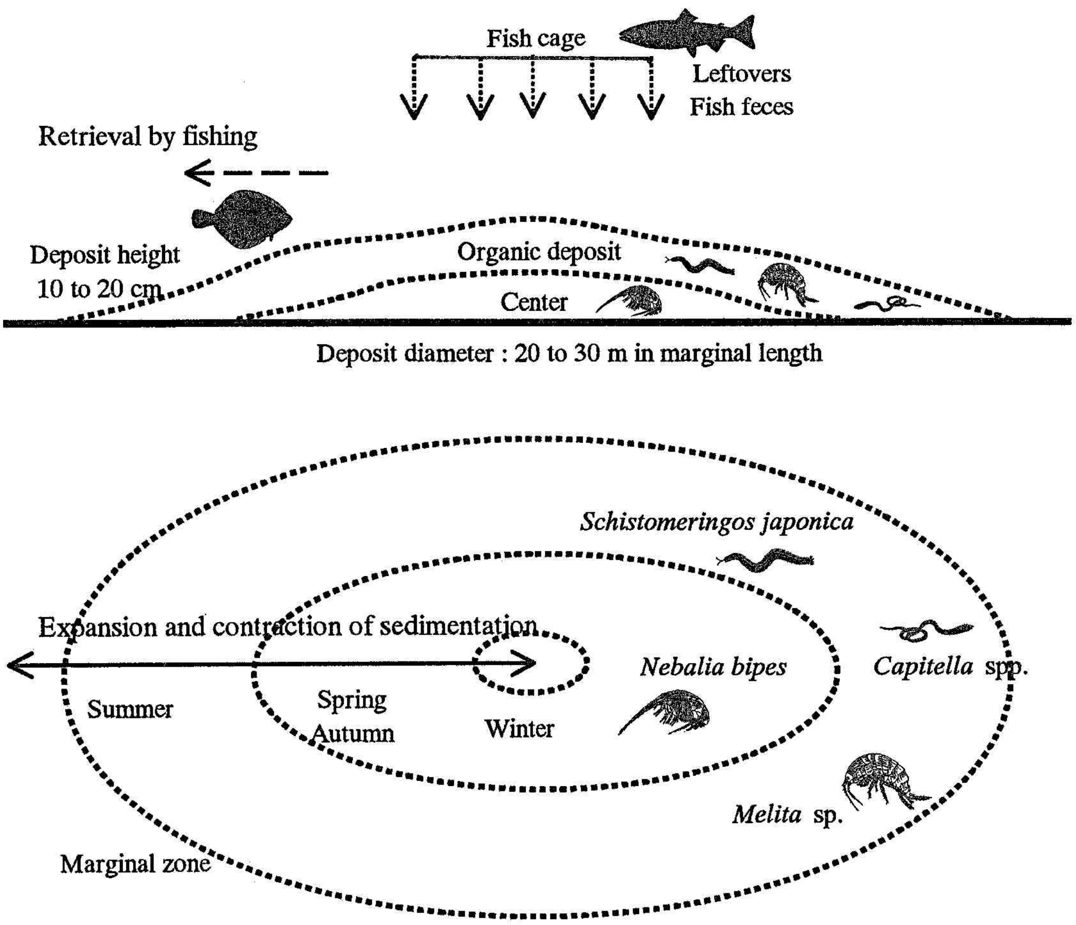

Fig. 12. Shematic diagram showing seasonal changes in sedimentation and aggregative distribution of the macrobenthos below coho salmon culture cage

of shell-collector was 13 individuals. At the same time, the number of *Nebalia bipes* collected oyster shell-collector suspended 10-cm distance above the bottom was 528 individuals. It was supposed that the suitable position of shell-collector was partially moated onto the bottom surface with enriched sediments. From the ecological viewpoint, the center area below culture cage was originally impoverished for macrobenthos by its environmental condition of bottom surface covered with enriched sediment, which was improved to the aggregative site for artificial habitat of *Nebalia bipes* set by shell-collector.

Concerning another species of macrobenthos, the average number of *Melita* sp. distributed within shell-collector at the center area was 59 individuals in summer and 453 individuals in winter. On the other hand, that without shell-collector in natural bottom sediment was almost nil at the same area below culture cage.

From monthly changes in the density of *Schistomeringos japonica* from May through July collected at the center area below culture cage, the average number of *Schistomeringos japonica* distributed within shell-collector was 1,503 individuals and that without shell-collector was 216 individuals, respectively, with mean density variance of 7 times. The adult number of *Schistomeringos japonica* was disappearing after August due to mortality attributed to reproduction.

Therefore, spreading shell-collectors regarded as a habitat for macrobenthos seems to be much more effective to promote the carrying capacity of macrobenthos below culture cage, which improve the decomposition processes of organic sediment.

Conclusion

According to direct diving observation below culture cage, it was evident that the organic sediments derived from food supply were deposited 10 to 20 cm in height and 20 to 30 m in diameter through harvest time, in which sediments were decomposing until following spring under natural environmental conditions (Fig. 12). Aggregative distribution of those macrobenthos was corresponded to the marginal zone of enriched sediments, which location was seasonally removed before and after harvest. High density of *Schistomeringos japonica* and *Nebalia bipes* were supported in accordance with organic sediments distributed around removing marginal zone below culture cage from spring to summer, and that of *Nebalia bipes*, *Melita* sp. and *Capitella* spp. were supported there from autumn to winter. On the contrary, very few macrobenthos appeared at the center of bottom surface below culture cage where enriched sediments were abundant. These faunal zones might be generally constructed by the bottom substratum for habitat based on the gradients of environmental conditions of organic loads that cause the differentiation in the concentration of oxygen in the bottom water and nature of the sediment. Ecologically these distributions of each macrobenthos, close correlation between aggregative site and organic sediments were supposed by nutrient linkage. From these findings observed in each seasons, temporal and spatial distribution pattern were recognized as a change of environmental conditions of bottom surface below culture cage. Distribution patterns of macrobenthos were concentrically removed from outer area toward the center point according to the synchronous contraction of decomposed organic sediments in marginal zone by benthic communities.

Considering to synchronous patterns of removal distribution mentioned above, biological activities of so-called bioturbation such as feeding, burrowing and gardening conducted by these macrobenthos were recognized important in preventing self-induced deterioration of fish-farming. In coastal and estuarine areas, infauna is known to affect physical, chemical and biological properties of sediment by bioturbation, namely its feeding, burrowing, tube building, defecation and ventilation activities (Kikuchi and Mukai, 1994). Tsutsumi and Montani (1993) has already pointed out that the

Capitella colonies increased rapidly and biological activities such as feeding, reworking etc. efficiently decomposed the organic matter added on the sediment. Besides these findings of *Capitella* spp., rearing experiment of *Nebalia bipes*, *Schistomeringos japonica* and *Melita* sp. would be needed to analyze the quantitative capacity of assimilation through decomposition processes of organic sediment.

A part role of decomposition and mineralization of the loaded organic matter was confirmed by bacteria and macrobenthos through cyclic processes under natural environmental conditions. Sulfate-reducing bacteria, *Beggiatoa* spp., was investigated in the bottom of coho salmon culture area and considered that occurrence and distribution of *Beggiatoa* spp. was regarded as a indicator of environmental conditions derived from the organic loads by food supply (Takekawa *et al.*, 1989). The significance in distribution of relationship between sulfur bacteria and macrobenthos would be furthermore needed to analyze the efficiency of decomposition processes of organic sediment.

In addition to natural decomposition carried out by those bacteria, it might be available that artificial habitat such as shell-collector set on the bottom surface promote the decomposition of enriched sediment through the activity of bioturbation with those species of macrobenthos. It is supposed that spreading shell-collectors as a habitat for macrobenthos on the bottom surface below culture cage seems to be effective for not only to promote the carrying capacity for decomposition of organic sediments but also to control eutrophicated conditions for improving the productivity of aquaculture biomass. From the ecological relationship between macrobenthos and sedentary fishes below culture cage, retrieval by sedentary fish propagated by macrobenthos with enriched sediment is a removal step for discharging the organic loads toward outside of natural ecosystem through food chain in the benthic community.

Future work should be focused on the goal with ecological basis for decomposition of organic sediment contributed by macrobenthos and on the relationship between sustainable carrying capacity and environmental conservation. The goal would be established by a biocontrol technology for planning mixed aquaculture that organized with algae as mineral assimilator, bivalvia as filter-feeder and fishes as organic loader in respect to the circulated eco-system through nutrient linkage.

Acknowledgments

We sincerely thank Prof. T. Ohmori (Tohoku University) for permission to use the data in Figure 3.

We are grateful to Dr. T. Miura (Kagoshima Univ.) for identification and information of *Schistomeringos japonica* and *Capitella* spp., Dr. Y. Kamihira (Hakodate Univ.) for that of *Melita* sp., Dr. T. Kikuchi (Yokohama Univ.) and Dr. R. Kado (Kitasato Univ.) for valuable information on *Nebalia bipes*. Thanks also to Dr. P. K. Park for improvements to the manuscript.

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Bull. Fish. Res. Agen. Supplement No. 1, 33-40, 2004

Influence of environmental changes in the tidal flats on the filtration and respiration of bivalve mollusks

Junya HIGANO*

Abstract Manila clam *Ruditapes philippinarum* and the other filter feeding bivalves in tidal flat are not only commercially important as seafood, but also ecologically significant because of their filtration activity. The volume of water filtration by bivalves in Ariake Sound is estimated to be equivalent to daily water exchange on the tidal flat in 1970s. However, the annual catch of Japanese littleneck has been decreased during the past 20 years in Japan. Especially, rapid decrease in the clam population in Ariake Sound since 1980s forced to depress the nationwide production. Recent coastal changes such as land reclamation, dike, port, barrage, and dam construction presumably brought about the environmental impact for filter feeding bivalves through water and sediment movement. Higher intertidal zone and supralittoral zone are intercepted by artificial structure such as dike and breakwater. Consequently suspended sediments are prevented from depositing at the higher intertidal zone and are drifted in littoral zone. High concentration of mud particles suppresses the water clearance of the clams. On the other hand, reduction of water current by barrages encourages the stratification. Hypoxia and anoxia often occur in subtidal zone of eutrophied sheltered coast under the stratified layer in summer. Complex effects of mud increase and oxygen shortage are considered to be physiologically harmful to filter feeding bivalves. The ecological function of tidal flat has been destroying and it disturbs the recovery of the bivalve resources.

Key words: bivalves, coastal change, filtration, Manila clam, reclamation

Transition on production of filter feeding bivalves in tidal flats

Many kinds of filter feeding bivalve mollusks inhabit in the tidal flat buried. Some of these are not only commercially important, but also ecologically important as a nutrient recycling. In fact, Manila clam, *Ruditapes philippinarum* Adams & Leeve, is the most abundant infaunal bivalve in tidal flat of Japan. Major production localities of Manila clam and the other commercially important filter feeding bivalves on sheltered inlets in Japan are listed as; Tokyo bay, Mikawa Bay, Ise Bay, Seto Inland Sea and Ariake Sound crossing southern half of Japan(Fig. 1). Fig. 2 shows the annual production of Manila clam in main prefectures and localities (Ministry of Agriculture, Forestry and Fisheries, Japan, Statistics and Survey Division, 1954-2001). There are three chronological stages in the clam production. The production from Tokyo Bay had occupied more than half of national production at the first stage in 1960s. At the second stage, the depletion of Manila clams in Tokyo bay was attributed to land reclamation in 1960s to 1970s, and

2003年６月12日受理 (Received on June 12, 2003)
* National Research Institute of Aquaculture, Fisheries Research Agency 422-1 Nakatsuhamaura, Nansei, Watarai, Mie 516-0193 Japan
Email: higa@fra.affrc.go.jp

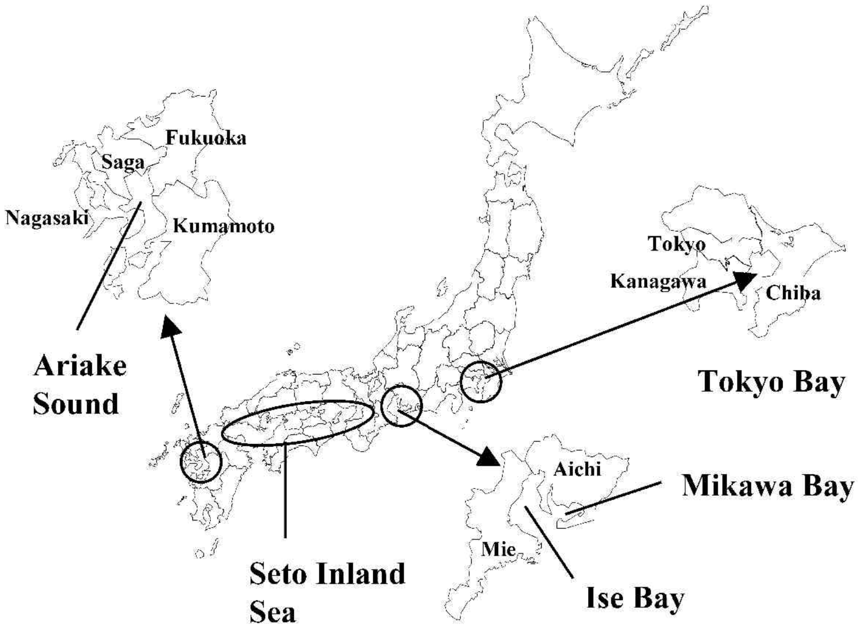

Fig. 1. Main bays and estuaries where *Ruditapes philippinarum* and other filter feeding bivalves inhabiting in their tidal flats

it was compensated by increase of the clam production in Ariake Sound that had the largest tidal flat in Japan. The annual catch had been maintained more than 100 thousand tons until mid 1987 since 1960. However, constant decrease of the clam in Ariake Sound since 1980s forced to depress even national production at the third stage. Only the production in Aichi Prefecture has been maintained through about 50 years. The other commercially important species of filter feeding bivalves has also decreased their production. Poker-chipped venus, *Meretrix lusoria*, is one of the most valuable clams in Japan, but decreased its production earlier (Fig. 3) than on Manila clam. National production of Poker-chipped venus has exceeded 10 thousand tons a year until 1966. It decreased very rapidly in 1970s by losing their habitat especially in Tokyo Bay. The clam was considered to be predominant before increasing the modern human activities because it is found dominantly in shell middens. It is difficult to explain the reason why *M. lusoria* had decreased so rapidly by environmental changes because there are few references (Kawamoto, 1967; Sagara, 1981) of environmental tolerance of *M. lusoria* than *R. philippinarum*. However,

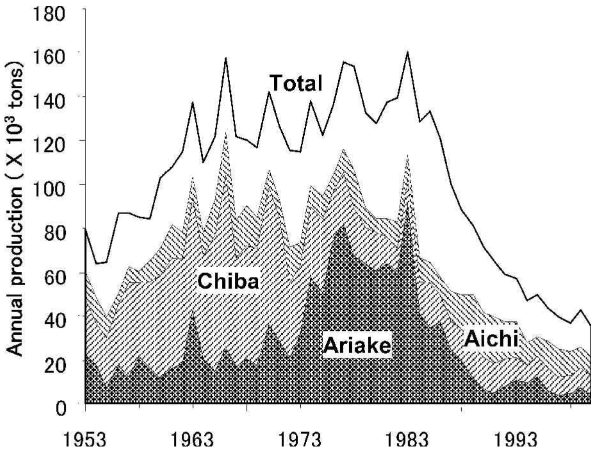

Fig. 2. Annual production of *Ruditapes philippinarum* in Japan; Aichi-Pref., Chiba-Pref. and total of four Prefs. in Ariake Sound

the fact that *M. lusoria* had started decreasing prior to Manila clam indicates this species prefers more natural habitat to have a dynamic estuarine circulation connecting the river mouth to tidal flat nearby.

There are several causes for explaining the rapid decrease of bivalve resources in tidal flat (Table 1). Over-catching is a commonly recognized cause on most of commercially important species inhabiting in the bottom sediment. Actually, the fluctuation of annual production of *R. philippinarum* and *M. lusoria* reflect the influence of over fishing and recovering by regulation of catch. The other causes are deeply concerned with environmental impacts by human activities. Physical alteration of coastal area may affect physical properties of habitats and chemical uses in watershed and sea area may cause the incompetence in their reproduction. Decrease of filter feeding bivalves must affect the coastal ecosystem by reducing their filtration.

Table 1. Causes of rapid decrease in filter feeding bivalves in tidal flats and its effects on the ecosystem

What are possible causes?
・Over catching
・Decrease of habitats
Land reclamation
・Change of food supply
・Change of bottom substrata
・Anoxic or hypoxic water
・Toxic chemical substances
・Disease and parasites
・Combining effects of hydrodynamics and organic load
What are main effects to the ecosystem?
・Decline of filtration capacity
・Change of nutrient cycle
・Increase of red tide
・Increase of oxygen deficiency in the substrata

Depression of filtration by bivalve mollusks

Manila clams and the other filter-feeding bivalves play an important role in purification of water through their filtering activity. Outbreak of exotic bivalves reportedly affected the phytoplankton density in the sheltered sea area such as San Francisco Bay (Alpine and Cloern, 1992). In Ariake Sound, frequency of red tide increased in 1990s whereas Tokyo Bay and Mikawa Bay have had the increase in 1970s-1980s. This is caused by not only eutrophication being connected to urbanization around the bays but also depression of grazing pressure by filter feeders.

Based on the fisheries statistics (Statistics and Survey Division, Ministry of Agriculture, Forestry and Fisheries, 1954-2001) and clearance rate of Manila clam estimated by Akiyama *et al.* (1986), I tried to estimate the daily clearance rate by filter feeding bivalves in Ariake Sound. In the calculation, it is assumed that the average harvested size was 35-40 mm in shell length which have specific clearance rates of 0.83 L/hr/g WW at 24 ℃ and 0.23 L/hr/g WW at 10 ℃, where WW is flesh wet weight. Ratio of flesh wet weight to total wet weight of Manila clam was averagely 0.4, thus total catch could be converted to the flesh wet weight by multiplying 0.4. For the bloody cockles and the other bivalves, 1.676 L/hr/g DW at 24 ℃ and 0.635 L/hr/g DW at 10 ℃ derived from the clearance rate of ark shell, *Scapharca broughtonii* (Fujiwara, 1986 and Yamamoto *et al.*, 1996), where DW is flesh dry weight. The duration of filtration by Manila calm assumed to be 22 hr a day as the average drying period at low tide was two hr. For the other bivalves, no drying period was considered.

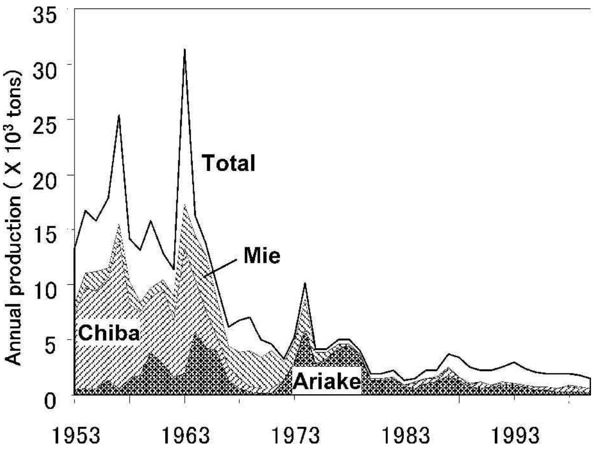

Fig. 3. Annual production of *Meretrix lusoria* in Japan; Mie-Pref., Chiba-Pref. and total of four Prefs. in Ariake Sound

Fig. 4 shows the annual change of daily clearance rate of bivalves inhabiting in Ariake Sound calculated under the condition that described above. This calculation contains many assumptions, but it is considered to approximate real situation. It fluctuates year by year and more than 300 million kL/day before 1990. But in recent decade it has decreased continuously and reached one tenth of the highest value in 1970s. Although total clearance rate by all bivalves was equivalent to daily water exchange rate on the tidal flat in spring tide (tidal rage is ca. 5 m) before 1990, that in 1996 to 2000 reached to only less than daily water exchange rate in neap tide (tidal rage is ca. 3 m) (Table 2). It is estimated that the clearance rate in winter is much less than daily water exchange rate in neap tide and nearly equal to daily river water discharge. Depression of clearance rate means the depression of all kinds of purifying and producing process by bivalves around tidal flat. It is connected to elimination of phytoplankton or other suspended matter, feces and pseudofeces production providing food for deposit feeders, retaining the nutrient to the bodies of bivalves and releasing inorganic nutrients to the water column enhancing primary production. The very low level of clearance rate in recent years presumably accelerates environmental deterioration in Ariake Sound.

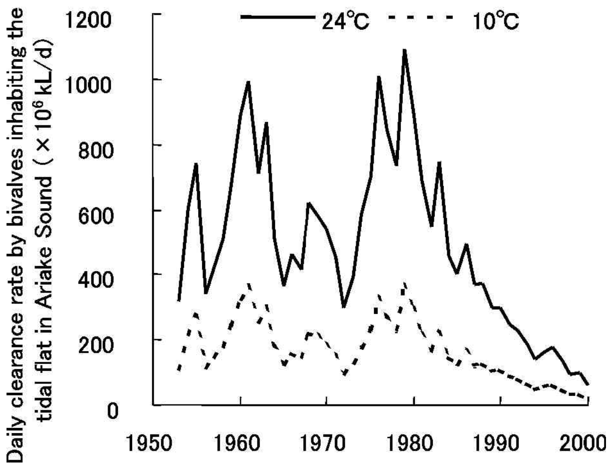

Fig. 4. Daily clearance rate of filter feeding bivalves in Ariake Sound calculated from statistical data of their annual productions (see text for calculation)

Coastal changes of sheltered sea area in Japan

Tidal flats in sheltered inlets or bays are formed by the relation between tidal current and sediment movement. As, in general, flood-tide currents are usually stronger than the ebb-tide currents, sediment moved into the inner estuary on the flood being left behind on the ebb (Little, 2000). Especially, at the upper part of the tidal flats tidal currents velocities are very low and finer sediments are deposited, then sediment in flats usually are sorted as finer at the higher than the lower parts (Little, 2000). Bloody cockles, *Anadara* spp. and the other filter feeding bivalves inhabiting higher intertidal zone may trap fine sediments through their biodeposition and contribute to forming tidal flats (Fig. 5).

In Japan, coastal line has been artificially changed for many years. Especially in sheltered bays, reclaimed lands and artificial struc-

Table 2. Comparison among total volume of Ariake Sound, water exchange, water discharge from rivers and clearance rate of bivalves (Unit : $\times 10^6$kL)

Total water volume		34,000	
Daily water exchange ontibal flat		Spring tide	Neap tide
	50 years ago	1,015	189
	Present	761	142
Daily discharge from rivers (average)		21.9	
Clearance rate by *R. philippinarum*		24°C	10°C
	Mid 1970s-mid 1980s	400-600	100-200
	1996-2000	23-51	6 -14
Clearance rate by Total bivalves		24°C	10°C
	Before 1990	400-1,000	100-370
	1996-2000	61-177	21-63

tures such as dikes, breakwaters and jetties have replaced natural coastal line. Reclaiming tidal flats is the easiest way in order to extend farmland, industrial zone and residential areas. Ariake Sound has been gradually reclaimed for a long time since the sixth century in accordance with natural land forming. After Meiji era (from 1868), huge undertaking of reclamation was started and the shoreline moved forward up to 5 km (Satoh, 2000). Tokyo bay was reclaimed at the very rapid rate after 1945 (Koike, 1990) and natural coastal line remains only 9.7 % whereas 19.6 % in Ariake Sound in 1984 according to Coastline Survey, 3rd National Survey on the Natural Environment (see: www. biodic. go. jp / reports / 3-2 / hyo/ w064_001. html). In other words, the pace of recent coastal changes such as reclamation in tidal flat, construction of dike, port, dam and barrage is very rapid and exceed the natural processes. It presumably brought about negative ecological impact for filter feeding bivalves through hydrodynamic changes of water and sediment movement. Higher intertidal zone and supralittoral zone are intercepted by artificial structure such as dike and break water. Consequently decreasing area of tidal flat caused the habitat decrease and increasing suspended mud by prevention of their depositing at the higher intertidal zone (Fig. 5). Dams and barrages often store the sand at the water reservoirs. It sometimes causes not only the decrease of reservoir capacity but also coastal erosion. Very fine sediment particles like silt and clay do not easily settle even in calm water as far as they are still in fresh water. In downstream region of dams and barrages, thus, fine sediments allow to discharge higher ratio in comparison with sand particles.

Both USA and Japan have a peak of dam construction around 1960 to exploit the energy and water supply (see: www. wec. or. jp/ center/ shiryou/ image/ download/ B-24.pdf). Japan still continued dam construction until 1990s mainly for water utilization despite USA put the brake on it after 1960s. This may have a possibility to make sediments in sheltered bays muddy and to change sediment property not suitable for originally inhabited bivalves.

Environmental Impact for filtration and respiration

How does coastal changes affect filter feeding bivalves? As I described above, coastal changes bring about the loss of habitat, depression of tidal current and accumulation of fine sediments. Fig. 6 indicates that these factors eventually affect the bivalves on both oxygen deficiency and high suspended load. Hypoxia and anoxia often occur in subtidal zone of eutrophied sheltered coast under the stratified layer in summer. Most of the bivalves can switch to anaerobicbiosis to maintain their lives and survive several days in anoxic condition (Hochachka, 1984; Nakamura *et al.*, 1997).

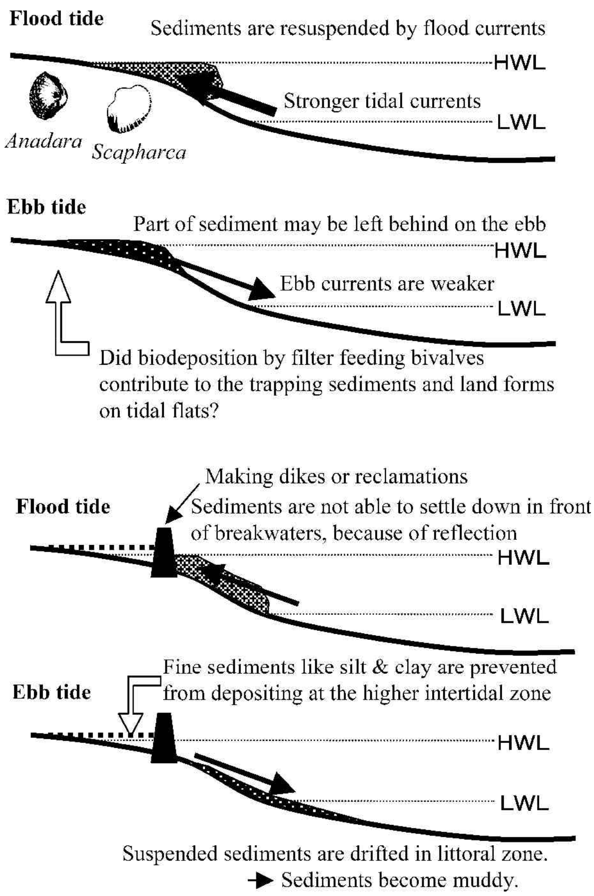

Fig. 5. Conceptional illustrations of land formation on upper level of tidal flat, and the impact of land reclamation and construction of artificial structure around high water level

However, elongation of anaerobicbiosis requires consumption of reserve substances like aspartic acid and glycogen. The accumulation of metabolites will indicate the limit to tolerate anoxia (Hochachka, 1984). Hydrogen sulfide is generated in anoxic layer of sediment and affects bivalves to inhibit aerobic respiration. Bivalves can also survive in relatively high concentration of hydrogen sulfide, but bivalves have to respond as same as under anoxia. Combined effects of hypoxia and hydrogen sulfide may act as anoxia, thus oxygen deficiency is extremely dangerous and should be noticed its effect on bivalve physiology.

High concentration of suspended particle suppresses the water clearance of the bivalves and increases pseudofeces ejection (Bayne, 1993). The increase of several tens mg/L of suspended mud mixed with food algae brings down the clearance rate and ingestion rate of food algae (Bricelj & Malouf, 1984). Increase of suspended particles may cause the excess energy expenditure for pseudofeces ejection and owe deficit on energy budget. The bivalves under the high turbidity should show low growth rate. Highly concentrated muddy water may clog the gill of bivalves and force to close the valves. The bivalves have to wait until the overlaying water replaced sufficiently clear for resuming respiration. So, high concentration of turbid water presumably affects the bivalves in almost same manner as anoxia.

Conclusions and further studies

Environmental changes in tidal flat in recent

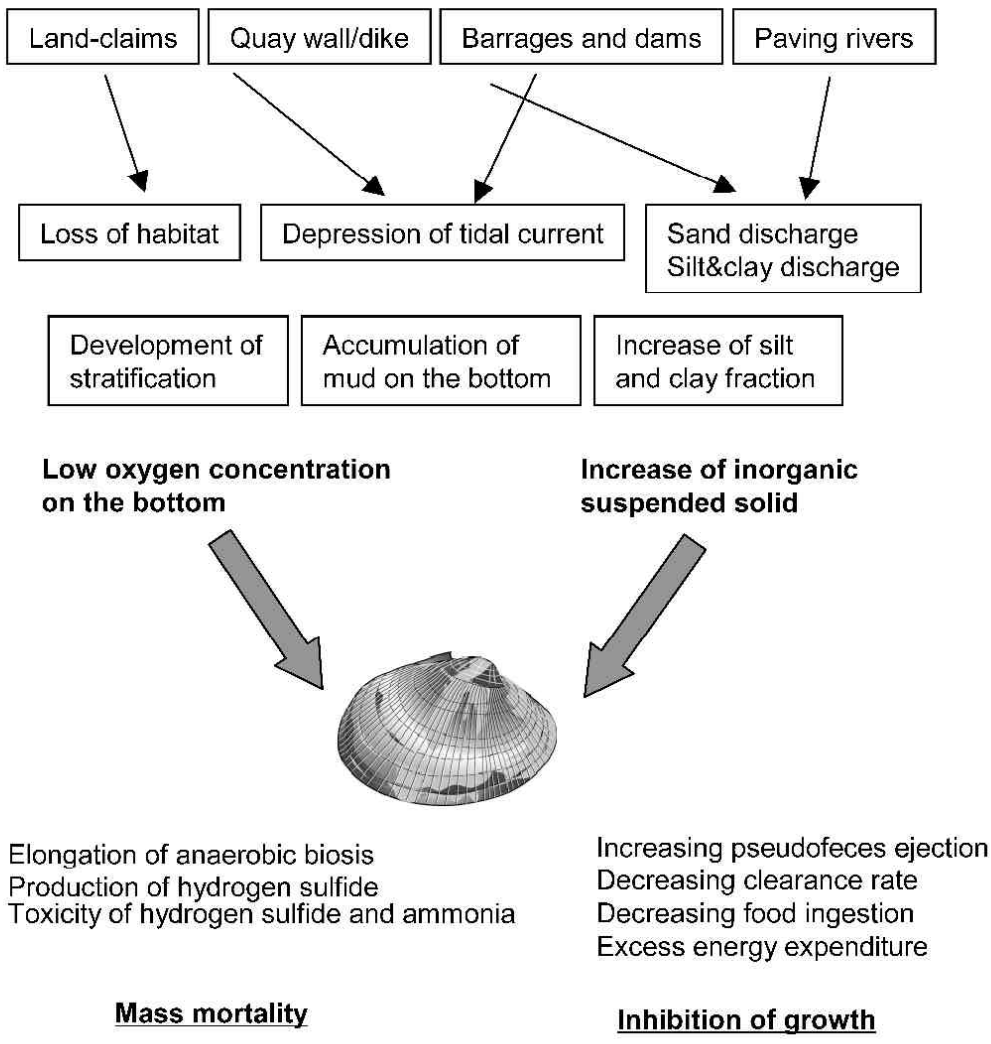

Fig. 6. Conceptual flow of coastal changes affecting to the depression of Manila clam resources

years probably affect the depression of the standing stock of filter feeding bivalves by high sediment load and oxygen deficiency. Consequently, the decrease of bivalve stocks may affect the surrounding environment. Once the ecological function of tidal flat has been degraded, it is very difficult to recover the proper bivalve resources. Under this situation, the natural recovery of ecosystem cannot be expected, and then the problem must be solved by human efforts. Quantitative assessments of causes and effects between environmental changes and bivalve's depression are strongly needed. Responses of the bivalves to single and compound environmental factors have to be clarified associating with their reproductive cycles. We also need to find out the suitable species for bioremediation. For example, the physiological and ecological evaluation of more tolerable species to turbid and anoxic condition will help design the habitat disposition. Availability of mechanical devices for improvement of water circulation and oxidation has to be considered at the same time. For example, the introduction of natural energy like wind and solar power to these devices and estimation of their cost-benefit should be inquired. Basically, efforts for eliminating negative factors and propagating the bivalve's stocks are essential to maintain coastal ecosystems.

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Bull. Fish. Res. Agen. Supplement No. 1, 41-50, 2004

The microbial loop in a eutrophic bay and its contribution to bivalve aquaculture

Takashi KAMIYAMA*

Abstract Information on planktonic food webs around bivalve farms is important, because bivalves utilize natural suspended matter as food. Not only phytoplankton but also other heterotrophic protists are occasionally essential to bivalves. Oysters cannot use bacterioplankton, but they can ingest protists that feed on bacterioplankton, thus using microbial energy indirectly through this microbial loop. To evaluate the importance of the microbial loop in planktonic food webs, occurrences of bacteria and heterotrophic protists were studied in the eutrophic Hiroshima Bay, where oyster aquaculture is economically important. Temporal changes in microbial loop components suggested that energy flow within the microbial loop was enhanced at the end of a phytoplankton bloom. The distribution of microbes and other protists implies that transfer efficiencies within planktonic food webs including the microbial loop differed among regions of the bay. Thus, the microbial loop may play an important role in planktonic food webs in Hiroshima Bay. In some oyster ponds in France, the importance of microbial energy flow to oyster production was actually evaluated. Possibly, energy flow within the microbial loop is also important to oyster production in Japan.

Key words: microbial loop, bacteria, flagellate, ciliate, oyster

In various oceanic regions, significant energy flows from bacteria to higher trophic levels and return flows from such organisms to dissolved organic matter pool, that is an original sense of the microbial loop (Azam *et al.*, 1983), have been clarified (eg. Sieburth, 1984; Pierce and Turner, 1992). In eutrophic bays, where the bacterial biomass and production are very high, possibly the bacterial production strongly influences planktonic food webs, which may enhance the productivity of higher trophic levels (Sherr *et al.*, 1986; Sherr and Sherr, 1988; Fukami *et al.*, 1999).

Hiroshima Bay is one of the eutrophic areas in the Seto Inland Sea of Japan. The northern, inner region of the bay is strongly influenced by riverine inputs and has a very low seawater exchange rate with the outer region. Also, like chlorophyll- *a* concentrations, the biomass and production of bacteria are high in the Seto Inland Sea (Yamaguchi *et al.*, 1995; Imai and Yamaguchi, 1996; Tada *et al.*, 1998), implying that microbial food webs play important roles in the ecosystem of the bay. There are many rafts for aquaculture of oysters throughout the whole bay and adjacent areas, and oyster production in these areas constitutes 50-60 % of the total Japanese production. To utilize the oyster productivity effectively and sustainably, it is necessary to clarify the food web components and the energy flow between them, including the microbial loop system.

In the present study, firstly, the temporal change of the microbial loop components in the coarse of a phytoplankton bloom was elucidated. Secondly, the characteristics of the

2003年６月12日受理（Received on June 12, 2003)
* Tohoku National Fisheries Research Institute, Fisheries Research Agency, Shinhama, Shiogama, Miyagi, 985-0001, Japan

distributions of the main microbial loop components [bacteria, heterotrophic nanoflagellates (HNF) and ciliates] as well as the environmental conditions in Hiroshima Bay in summer were evaluated. Thirdly, the function of the microbial loop to oyster production was reviewed and then the importance of the energy flow from ciliates to oyster was discussed.

Temporal changes in the microbial loop components in the coarse of a phytoplankton bloom

The investigation was conducted in a harbor (ca. 5-m water depth) in the northern part of Hiroshima Bay, the Seto Inland Sea of Japan (Fig. 1), to clarify the temporal changes in the microbial loop components in the coarse of the harmful alga *Heterosigma akashiwo*. Seawater sampling was carried out at surface and 1 m above the bottom every one to four days in the mornings from 6 June to 17 July in 1995. *Heterosigma akashiwo* cells were counted in fresh seawater from each depth. A seawater subsample was fixed with Lugol's iodine solution (final concentration : 2 %), and then concentrated by settling. Microzooplankton (zooplankton with body widths less than 200 μm) of each subsample were counted in 1-2 mL of concentrated samples with a phase contrast microscope using a Sedgwick-Rafter chamber. Another seawater in each sample was fixed by glutaraldehyde (final concentration: 1 %), and fixed bacteria and HNF were stained with DAPI (Porter and Feig, 1980) and DAPI and FITC (Sherr and Sherr, 1983a; 1983b), respectively, and then counted with epifluorescence microscopy.

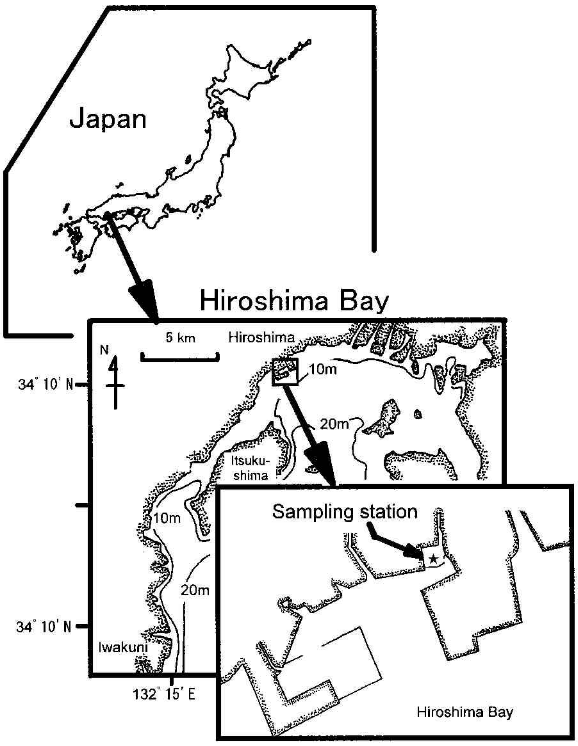

Fig. 1. Sampling station for investigating the change in the microbial loop components in a harbor in Hiroshima Bay, the Seto Inland Sea of Japan

Decay of tintinnid-ciliate population

A *H. akashiwo* bloom with a cell density of over 10^4 cells mL^{-1} in the surface water occurred from 23 June to 1 July 1995 (Fig. 2). A typical negative effect of the *H. akashiwo* bloom on the microzooplankton community was the decay of tintinnid ciliate population. During the bloom period, the abundance of tintinnid ciliates in the surface layer markedly decreased by one order of magnitude, compared to the abundance before the bloom (Fig. 2). The species diversity (Shannon-Wiener's H') of tintinnids in the surface layer also decreased during the bloom. Similar phenomena during *H. akashiwo* blooms have been reported in previous studies (Verity and Stoecker, 1982; Kamiyama, 1995).

Response of the microbial loop components

The microbial loop components fluctuated in response to the formation and decay of the bloom. Two abundance peaks of bacteria in surface water were recorded just after the beginning and just after the end of the bloom (Fig. 3). Also, the fluctuations in HNF abundance were found to be tightly coupled with those of bacterial abundance; there were two abundance peaks detected after 1 - 3 days of the bacterial abundance peaks. Although conspicuous change of aloricate ciliates was not observed during the process of bloom formation, the abundance increased drastically at the end of the bloom, and temporarily became two orders higher in magnitude than before the bloom.

These fluctuations of the microbial loop components reveals a drastic change in the food web system during the course of the *H. akashiwo* bloom. In particular, it is clear that the decay of the bloom enhanced the flow of energy within the microbial loop from the bacteria through the HNF to the aloricate ciliates. This process is interpreted as follows. The decay of the *H. akashiwo* bloom probably caused increase in dissolved organic matter (DOM) because of cell lysis of *H. akashiwo*. Increased DOM possibly stimulated the production of heterotrophic bacteria (Cole *et al.*, 1988). Consequently, bacterivorous organisms such as HNF and small aloricate ciliates increased in response to the food supply. Partly, production of aloricate ciliates may be also promoted by the increase of HNF. It is interesting that it took only a few days for the process from decay of the bloom to the increase of aloricate ciliates. The microbial loop system probably responds the change of the plankton community quickly.

Characteristics of the distributions of bacteria, HNF and ciliates in Hiroshima Bay in summer

Investigation was performed in 4 - 6 layers in the water column at 6 sites in Hiroshima Bay (Fig. 4) from a research vessel in summer, (13-15 June and 20-22 August 1996, and 1 - 3 July 1997). Environmental parameters were measured and analyzed (temperature, salinity, chlo-

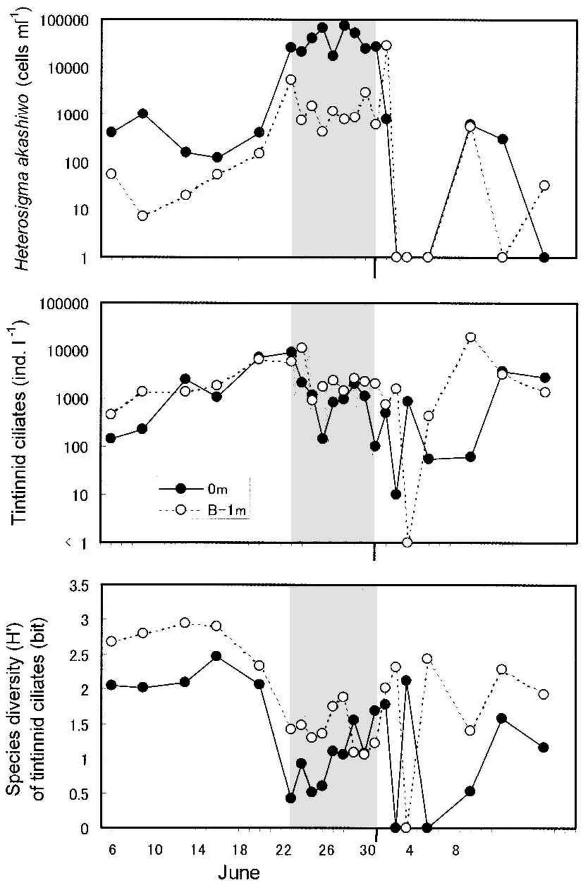

Fig. 2. Temporal changes in abundance of *Heterosigma akashiwo*, and abundance and species diversity of tintinnid ciliates during the course of the bloom. Shaded areas indicate the bloom period (23 June to 1 July 1995) when the density of *H. akashiwo* exceeded 10^4 cells mL^{-1}

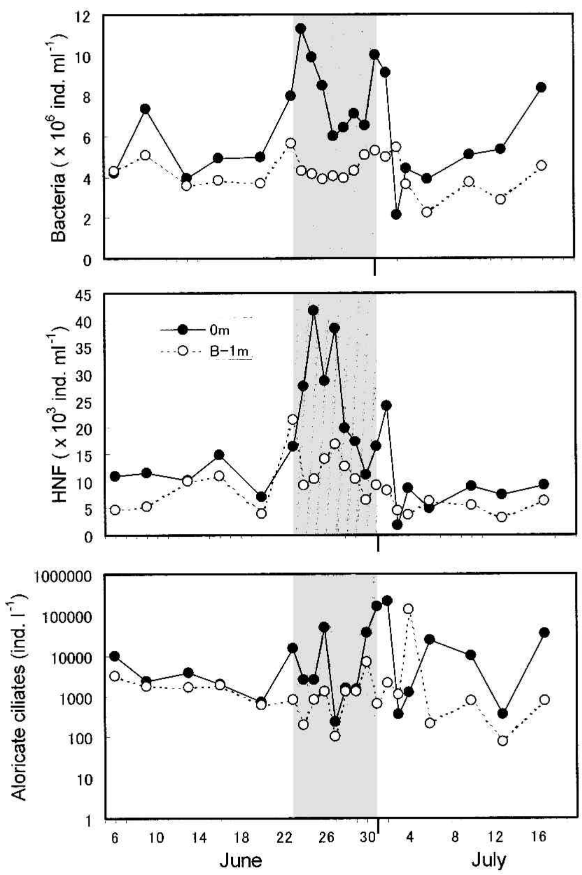

Fig. 3. Temporal changes in the abundance of microbial loop components of bacteria, heterotrophic nanoflagellate (HNF) and aloricate ciliates during the course of a *Heterosigma* akashiwo bloom. Shaded areas indicate the bloom period (23 June to 1 July 1995) when the density of *H. akashiwo* exceeded 10^4 cells mL^{-1}

rophyll-*a* and nutrients). Based on unfractionated seawater (total faction) and the seawater filtered through a 20-μm mesh screen (<20-μm fraction), the chlorophyll-*a* concentration in each fraction was measured with a Turner Designs fluorometer according to Suzuki and Ishimaru (1990). From another filtered seawater sample, the concentrations of dissolved organic nitrogen (DIN) that consists of NO_2-N, NO_3-N, NH_4-N, and PO_4-P and SiO_2-Si were determined using a TrAAcs 800 autoanalyzer (Bran-Luebbe Co.)

The main microbial loop components (bacteria, HNF and ciliates) were also investigated at 3 of the 6 sites: the inner (Stn. 1), central (Stn. 3) and outer (Stn. 5) regions of the bay (Fig. 4). Abundances of them were generally measured with the same method as the above. Simultaneously the cell dimensions of each taxon for HNF and aloricate ciliates and the lorica dimension of each tintinnid-ciliate species were measured, and the cell volume of them was calculated by approximating the shape of each taxon to a standard geometric configuration. Bacterial carbon biomass was estimated based on the average cell volume (0.098 μm^3 $cell^{-1}$) from annual Hiroshima Bay data (Imai and Yamaguchi, 1996) and an allometric conversion equation (29.6 f gC $cell^{-1}$; Loferer-Krößbacher *et al.*, 1998). The carbon biomass of HNF and aloricate ciliates were estimated based on the cell volume data and the carbon-volume conversion factors for HNF (0.22 pgC $cell^{-1}$; Børsheim and Bratbak, 1987) and for aloricate ciliates (0.19 pgC μm^{-3}; Putt and Stoecker, 1989), and that of tintinnid ciliates were calculated based on the volume of the lorica according to Verity and Langdon (1984).

Environmental conditions

The strong influence of riverine inputs on the seawater environment in the inner region of the bay was recognized by the low salinity in the surface on all occasions. Similarly, in July 1997, the concentrations of nutrients (DIN, PO_4-P and SiO_2-Si) were comparatively high in the surface layer at the innermost site (Stn. 1; distribution of only DIN concentration shown in Fig. 5). However, high concentrations of DIN in the surface layer were limited to the innermost site where chlorophyll-*a* concentrations were high (Stn. 1, 2), suggesting that riverine-nutrient inputs may rapidly be taken up by phytoplankton in the vicinity of the river mouth areas in Hiroshima Bay. Generally, higher concentrations of chlorophyll-*a* were recorded in the surface and near-surface layers in the inner regions of the bay (Stn. 1 and 2; Fig. 6). The contribution of the <20-μm fraction to total chlorophyll-*a* concentration mostly exceeded 60 % in June 1996 and in July 1997. In August 1996, also more than 60 % of the contribution of the <20-μm fraction was recorded in the almost all layers at Stn. 1 and 2 although less than 40 % of the contribution was partly

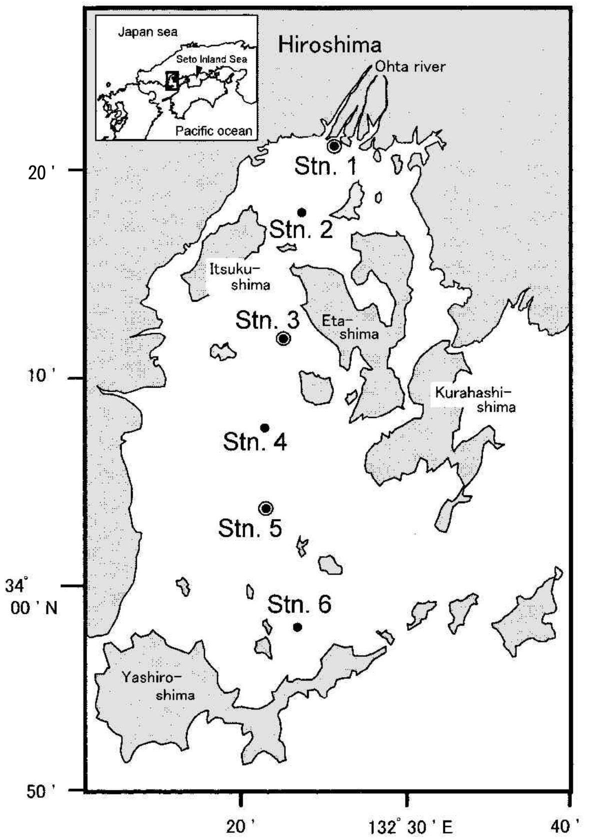

Fig. 4. Study sites in Hiroshima Bay for investigating the distribution of the microbial loop components. Environmental conditions were surveyed at all sites, while abundance and biomass of the microbial loop components (bacteria, heterotrophic nanoflagellates and ciliates) were investigated at Stn. 1, 3 and 5 only (◉)

observed in the central and outer regions of the bay. This result indicates that the phytoplankton assemblage is generally dominated by nano- and picoplankton in the inner region of the bay in summer.

Abundance of the main microbial loop components

In the inner region of the bay, abundances of the main microbial loop components were higher than in the other regions. Abundances of bacteria and HNF ranged from 1.10×10^6 cells ml^{-1} to 5.75×10^6 cells ml^{-1} and from 0.52×10^3 cells L^{-1} to 11.06×10^3 cells L^{-1}, respectively. The highest abundances of both organisms on each occasion were recorded in the surface, 2-m or 5-m layers at Stn. 1 (Fig. 7). The abundance of total ciliates ranged from 0.6×10^2 ind. L^{-1} to 20.5×10^3 ind. L^{-1} during the study, and distributed abundantly in the surface and 2-m layers at Stn. 1 in all months. Ciliate assemblages were generally dominated by aloricate forms except for samples collected in August 1996 at Stn. 1 and 5 (Fig. 8). In July 1997, the maximum abundance of ciliates, especially the tintinnid forms at Stn. 1, was considerably lower than in June and August 1996 (Fig. 8).

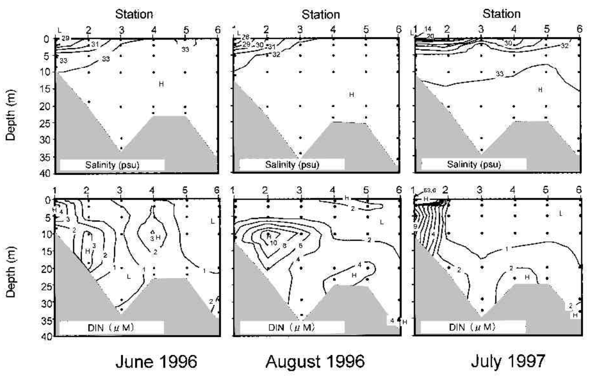

Fig. 5. Horizontal and vertical distributions of salinity and dissolved inorganic nitrogen (DIN) concentration.

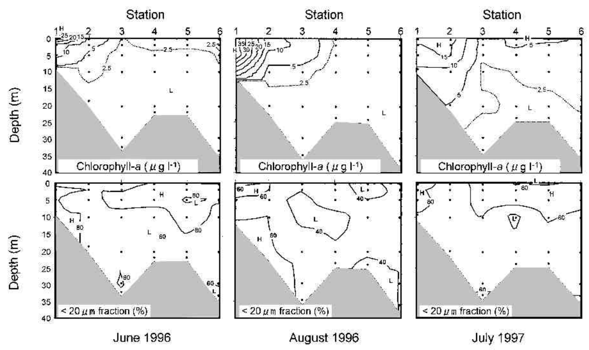

Fig. 6. Horizontal and vertical distributions of the chlorophyll-*a* concentration and the percentage of the chlorophyll-*a* concentration accounted for by the <20-μm fraction

Transfer efficiency from primary producers to their grazers

Among Stn. 1, 3 and 5 in the present study, Stn. 1 was characterized by high chlorophyll-*a* concentrations and high abundance of the microbial loop components. High biomass of primary producers may sustain the high production of the grazer plankton. Further, to evaluate the transfer efficiency of energy from the primary producers to their grazers, the relationships of the carbon biomass between each ciliate assemblage (tintinnid or aloricate ciliate) and the prey organisms (bacteria, HNF, and <20-μm chlorophyll-*a*) were analyzed, assuming that the carbon conversion factor of the <20-μm chlorophyll-*a* was 30 (Riley, 1965; Parsons, 1984). For this analysis, stable prey-predator conditions are necessary but at Stn. 1 in July 1997, environmental conditions indicat-

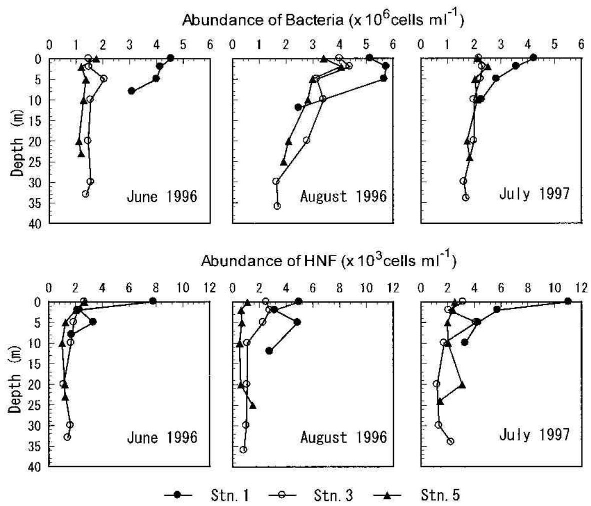

Fig. 7. Vertical distributions of the abundances of bacteria and heterotrophic nanoflagellates (HNF) at Stn. 1, 3 and 5 in Hiroshima Bay

ing extremely low salinity from riverine inputs imply that the stable prey-predator conditions was not applicable for the ciliate assemblage. Hence, data from Stn. 1 in July was not used for the analysis.

Regarding the relationships between aloricates and the total prey organisms, the significant positive linear correlations ($r^2 = 0.45 - 0.73$, $p < 0.05$) were observed at all sites (Fig. 9). The slope of the linear regression at Stn. 3 was significantly higher than those at Stn. 1 and 5 ($p < 0.01$). Further, the X-axis intercept at Stn. 1 in Fig. 9 was significantly higher than those at Stn. 3 and 5 ($p < 0.01$), implying that the biomass of primary producers and microbes not utilized by aloricate ciliates was higher at Stn. 1 than at the other sites. These interpretations demonstrate that the energy transfer efficiency from primary production and microbes to aloricate ciliates may be low in the inner region of the bay (Stn. 1) compared to the central region of the bay (Stn. 3).

To consider the energy transfer efficiency, it is necessary to practically measure the primary production and subsequent grazing impact, because information on biomass only represents a scene of dynamic relationships between prey and predator. In spite of such limitations, biomass relationships between prey and predators in this study were clear and characteristics differed among areas in the bay, suggesting that the biomass relationship may be one of the factors to evaluate the efficiency of energy flow in planktonic food webs.

Contribution of the microbial loop to production of bivalve aquaculture

The size of prey that bivalves can feed upon depends on the bivalve species. Some mussels can retain the particles of bacterial size (Kreeger and Newell, 1996) but oysters cannot efficiently utilize the picoplankters (Langdon and Newell, 1990). However, the oysters have the ability to efficiently retain the particles larger than 6μm (Palmer and Williams, 1980), which included the size of bacterivorous protists. Hence, it is a possibility that oysters can utilize the picoplanktonic energy source by ingesting bacterivorous protists. Le Gall *et al.* (1997) proved this scenario. The cyanobacteria *Synechococcus* sp. retaining specific yellow-gold autofluorescence was supplied for the ciliate *Uronema* sp. as food source. This protist that

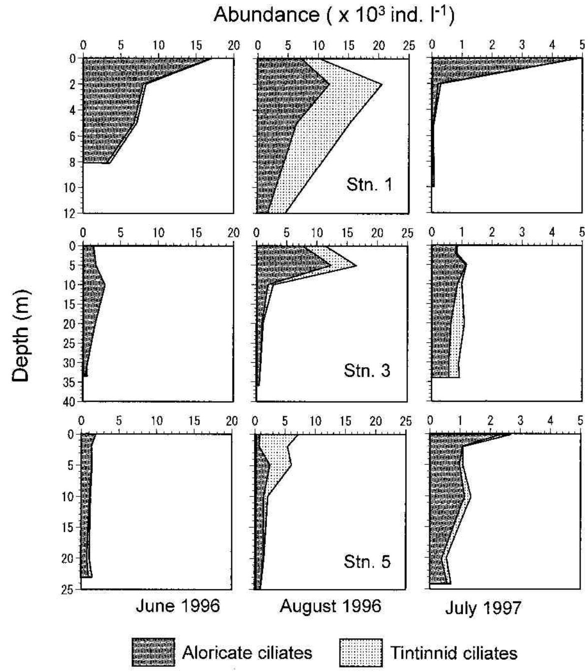

Fig. 8. Vertical distributions of the abundance of ciliates at Stn. 1, 3 and 5 in Hiroshima Bay

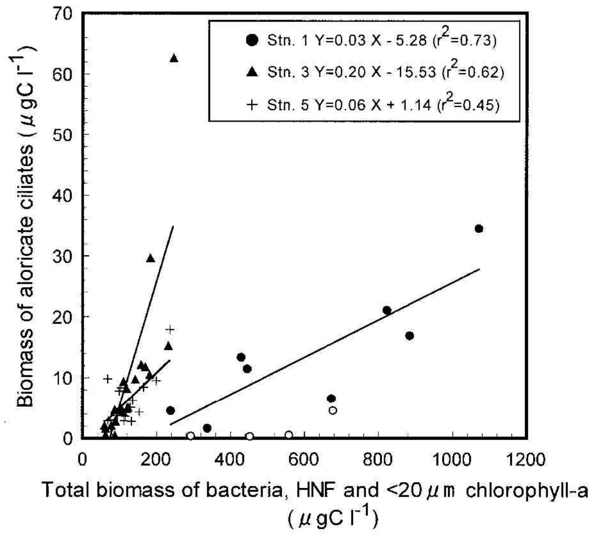

Fig. 9. Relationships between the carbon biomass of aloricate ciliates and all prey organisms that consist of bacteria, heterotrophic nanoflagellates (HNF) and <20-μm fraction of chlorophyll-*a*. ○ (data at Stn. 1 in July 1996) was omitted from the calculation of the regression line

fed on *Synechococcus* was labeled by many spots of the yellow-gold autofluorescence observed by epifluorescence microscopy under blue-light excitation. Then, the labeled *Uronema* was supplied to oysters, large amount of autofluorescent particles clumped on residual membranes of labeled ciliates was observed in the stomach of oysters, although few parcels could be found in the stomach of oysters which were provided with only *Synechoccus* suspension. The results suggest that the ciliates play a role as a trophic intermediation between picoplankton and oysters. This result is evidence that oysters can receive from not only phytoplankton energy source but also from the microbial loop energy flow.

Actually, the effects of oyster feeding on protists and picoplankton were compared under laboratory experiments (Dupuy *et al.*, 2000b). While the abundance of picophytoplankton was constant under both conditions with a filtering oyster and without oyster, in the case of ciliates as prey, the abundance decreased under the conditions with a filtering oyster for the first 15 minutes (Fig. 10). Also, the feeding activity of oysters was compared between food sources. The clearance rate of oysters on ciliates was in the same level as that on diatoms as shown in Fig. 11 (Dupuy *et al.*, 2000b). Furthermore, based on the feeding activities of the oyster *Crassostrea gigas* on the ciliates, the carbon specific feeding impacts of oysters on ciliate assemblages in some oyster ponds in France were estimated and compared with the impact on phytoplankton assemblages (Table 1). The results indicated that the ratio of removed carbon contents from ciliate assemblage to that from phytoplankton assemblage ranged from 0.30 to 4.5, implying that importance of ciliates for oyster probably depends on the environmental conditions and the food-web systems in the oyster farming area. Ciliate may not be always important food source for oysters, but there is a possibility that they play an important role as a food source if the microbial loop activity was high and the phytoplankton biomass is insufficient for oyster food.

The role of the heterotrophic protists as food source for oysters has been pointed out in the some ponds where the oyster was cultured on the bottom (Dupuy *et al.*, 1999; 2000a; 2000b). In Japan, generally cultured oysters are suspended into the seawater from the specific rafts or line to get higher production of oyster in a unit area. Hence, probably the oyster production more strongly depends on the planktonic food webs in the oyster farming areas. In some areas in Japan, unknown death in summer, decreasing production in a unit area and damage due to red tides have caused many problems for

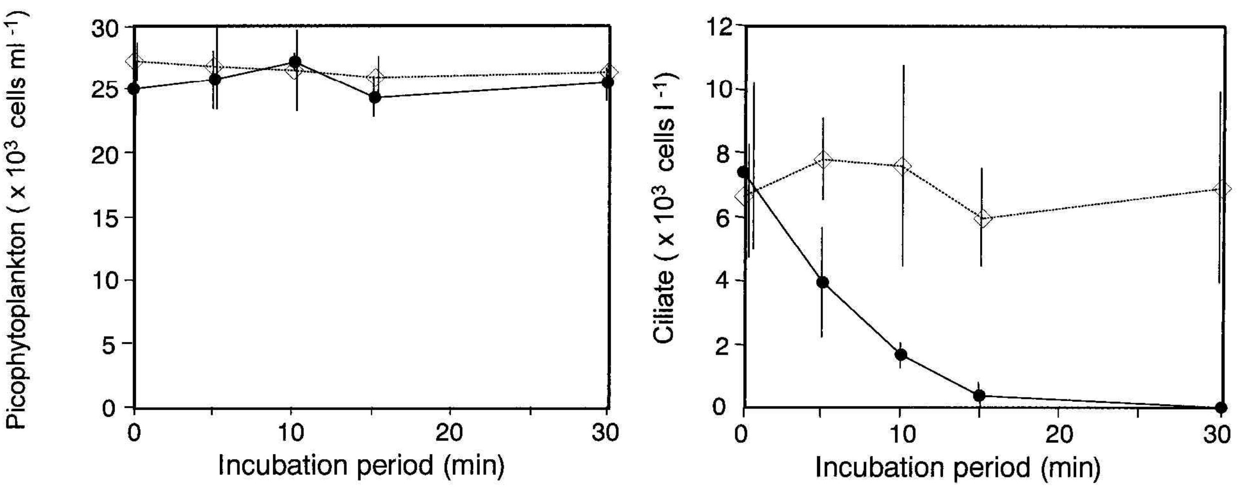

Fig. 10. Change in abundances (mean±SD, n=3) of picophytoplankton and ciliates under the conditions with a filtering oyster (●) and without oyster (◇)(Modified from Dupuy *et al.*, 2000b)

Table 1. Feeding impact by *Crassostrea gigas* on ciliates and phytoplankton

Area	Ciliates			Phytoplankton			A/B
	Prey	Biomass (μgC L^{-1})	Removed (A) (μgC $h^{-1}g^{-1}$)	Prey	Biomass (μgC L^{-1})	Removed (B) (μgC $h^{-1}g^{-1}$)	
French Atlantic coastal ponds*[1]	FA	63.5	126	CD	–	27.5	4.5
French Atlantic coastal ponds*[2]	FA	7-32	48-218	FA	161-648	641-2652	0.07-0.21
Mediterranean Thau Lagoon*[3]	FA	3	38.6	FD	161.5	1307	0.03

*[1]: Dupuy et al. 1999, *[2]: Dupuy et al. 2000a, *[3]: Dupuy et al. 2000b.
FA: field assemblage, FD: field diatoms, CD: cultured diatom (*Phaedactylum tricornatum* 1×10^6 cells L^{-1})

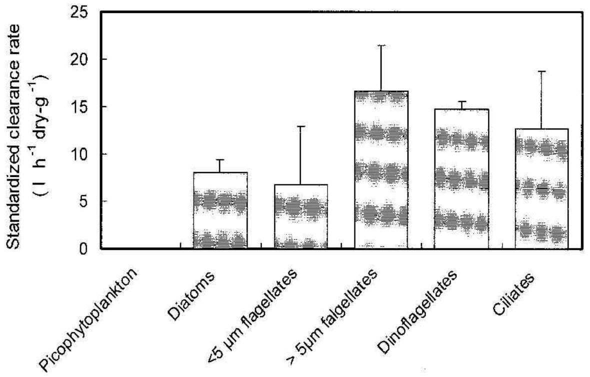

Fig. 11. Standardized clearance rates (mean ± SD, n=3) of the oyster *Crassostrea gigas* on the various taxonomic groups. Data taken from Dupuy *et al.* (2000b)

oyster aquaculture. One of the reasons is too high density of oyster in the farming area, which probably exceeded the carrying capacity for oyster cultivation suitable for the particular environmental conditions. To use the bivalve farming area efficiently and lastingly, it is essential to estimate the carrying capacity for oyster aquaculture. For that, it is necessary to quantitatively clarify the food-web system (biomass of each planktonic group, its production and the trophic interaction) and to monitor environmental characteristics in oyster farming area as well.

Acknowledgements

The author thanks Drs. Itakura and Nagasaki, National Research Insitutute of Fisheries and Environment of Inland Sea (FEIS), for their cooperation during the field investigation for the *H. akashiwo* bloom. Thanks are also extended to Mr. Arima and Ms. Tsujino, FEIS, and the captain and all the crew of the R/V Shirafuji-Maru, for their assistances of field investigation of Hiroshima Bay.

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Balancing marine aquaculture inputs and extraction: Combined culture of finfish and bivalve molluscs in the open ocean

Richard LANGAN*

Abstract Enrichment of the water column with dissolved nutrients and of bottom sediments with organic matter as a result of culturing finfish in sea cages have been identified as real and potential environmental impacts of fish culture. While severe impacts have been documented in shallow, poorly flushed waters, proper siting of sea cage operations generally results in only minor localized impacts to the benthic community on the sea floor directly beneath the cages. None the less, the perception of environmental groups and regulatory agencies in the U.S.A. that fish waste and uneaten feed will impact the marine environment regardless of siting has affected the expansion of existing sites and the establishment of new sites. In order for the industry to expand to meet the growing demand for seafood, measures to mitigate these impacts must be taken. One possible solution is to balance inputs of feed with extraction of biomass of organisms such as marine plants and bivalve molluscs that do not require external feed application.

In 1999, the University of New Hampshire established the Open Ocean Aquaculture Demonstration Project. Funded by the National Oceanic and Atmospheric Administration, the project was designed to provide a commercial scale demonstration and research site for open ocean aquaculture in the northeast U.S.A. The project is an integrated, multi-disciplinary, regional effort that includes biology, oceanography, engineering, sociology, economics, technology transfer, and education. While the development of technologies for finfish and shellfish production in offshore environments is central to the mission of the project, demonstration of the environmental sustainability of open sea culture is critical to the social acceptance of industry development.

Since 1999, the project has produced harvests of several species of finfish using submersible sea cages and six crops of molluscan shellfish (primarily blue mussels) using submerged longlines in close proximity to the sea cages. While not considered true polyculture, the harvest of the filter feeding bivalve molluscs represents a net removal of nitrogen, carbon and phosphorus that can be used in mass balance to offset the addition of these nutrients from finfish feeding. In this paper, data the potential for balancing inputs associated with feed application and fish wastes with extraction of fish and bivalve biomass will be examined.

Key words: bivalve mollusc culture, finfish culture, sea cages, waste feed, nitrogen

2003年６月12日受理（Received on June 12, 2003）
*[1] Cooperative Institute for New England Mariculture and Fisheries, University of New Hampshire, Environmental Technology Building, 35 Colovos Road, Durham, NH 03824, USA, Email: rlangan@cisunix.unh.edu

As the world's human population continues to grow, the demand for food, and especially protein, increases correspondingly. The protein derived from seafood accounts for 13 % to 16 % of the human diet worldwide. In other words, in order for high quality protein production to keep pace with population growth, seafood production must increase. Addressing this shortage of seafood requires more production from either capture fisheries or aquaculture. By most accounts production from capture fisheries is unlikely to increase from the current levels (FAO, 1998), therefore aquaculture will become an increasingly important source of seafood production.

Marine aquaculture systems are quite diverse, ranging from highly controlled land-based systems that recycle water and remove and treat wastes to open water cages that release wastes directly to the environment. Species produced in the marine environment are also very diverse, and include seaweeds, bivalve molluscs, echinoderms, crustaceans, and finfish. As with all concentrated food production systems, aquaculture can potentially have negative impacts on the environment, however, the severity of impacts depends on the culture system employed, the location, the species under cultivation, and husbandry practices. In the marine environment, negative impacts have been attributed to all forms of aquaculture, though finfish aquaculture has been the primary target of most of the critics of the aquaculture industry (Goldberg and Tripplett, 1997). One of the negative effects attributed to finfish culture is enrichment of the water column with dissolved nutrients, resulting from the decomposition of uneaten feed, and from metabolic wastes produced by the fish (Fig. 1).

While the amount of dissolved nitrogen discharged from fish farms may be locally significant and could potentially impact poorly flushed shallow embayments by increasing phytoplankton production, the volume of the discharge relative to other human sources of nitrogen to coastal waters is negligible (McVey *et al.*, 2002). In well flushed areas, it is unlikely that nitrogen addition from finfish aquaculture would have any impact on trophic conditions of the receiving waters (Sowles and Churchill, 2002). None the less, the perception of environmental groups and regulatory agencies in the U.S.A. that fish waste and uneaten feed will impact the marine environment regardless of siting has affected the expansion of existing sites and the establishment of new sites. In order for the industry to expand to meet the growing demand for seafood, measures must be taken to address these environmental concerns.

The salmon industry worldwide has greatly reduced nitrogen discharge by developing better feed formulations, reducing the amount "fines" or small feed particles by improved feed handling and delivery methods, and by monitoring the feeding behavior of the fish with video systems and ceasing feed delivery when the fish stop feeding (Sowles and Churchill, 2002). Another possible solution is to balance inputs associated with fish feeding with extraction of biomass of organisms such as marine plants and bivalve molluscs that do not require external feed application. This approach has been referred to as integrated aquaculture and is attracting increasing attention as a way to mitigate the real and perceived impact of finfish culture (McVey *et al.*, 2002 ; Chopin *et al.*, 2002).

The concept and conduct of polyculture or integrated aquaculture is not new, and has been

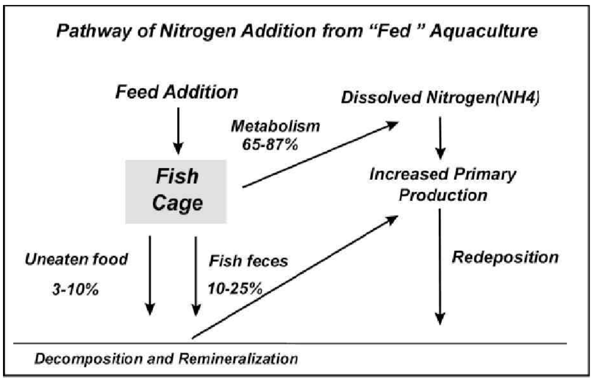

Fig. 1. Pathway of nitrogen wastes generated by the addition of fish feed in open water systems

practiced for centuries in Asian countries (Chopin *et. al.* 2002). While the rationale for considering integrated aquaculture today concerns mitigation of nitrogen additions from fed aquaculture, the result is similar, in that one species benefits from the waste products of another. With dissolved nitrogenous wastes, plants such as seaweeds benefit with enhanced growth from the increase in essential nutrients. Harvesting plant biomass for food or for pharmaceutical or industrial extracts constitutes mitigation of the nitrogen added through feed addition. Bivalve molluscs can also incorporate the waste nitrogen, however the pathway is indirect, relying on an increase in phytoplankton production resulting from the nitrogen additions (Yarish *et al.*, 2002).

Integrating seaweed or bivalve culture with finfish culture provides a mechanism for removing some of the added nitrogen resulting from feed introduction through harvest. The pathway of waste nitrogen in integrated systems is illustrated in Fig. 2.

The effectiveness of extractive aquaculture species in mitigating nitrogenous wastes produced by finfish culture has been demonstrated for closed-system, intensive land based culture (Shpigel *et. al.*, 1993). Obtaining accurate, quantitative mass balances for waste addition and removal are much more difficult in open water systems since hydrographic conditions, natural and analytical variability, and seasonal changes in fish physiology make it difficult to isolate changes in water quality due to aquaculture. A more simplistic a approach would be to calculate the amount of nitrogen discharged to system from finfish operations, and the amount of nitrogen extracted by plants or bivalves in harvested biomass. In 1999, the University of New Hampshire established a 30-acre aquaculture demonstration site 8 km from shore in the open waters of the Gulf of Maine (Fig. 3). The site located in 52 m of water and is fully exposed to wind and tides from all directions. Wave heights can reach 9 m in severe storms.

Aquaculture installations at the site include two submersible fish cages and two submerged longlines for bivalve mollusc culture. While the project was not intentionally established to demonstrate integrated aquaculture, the presence of finfish and bivalve molluscs provided an opportunity to investigate the capacity of the molluscs to mitigate nitrogen wastes resulting from finfish production.

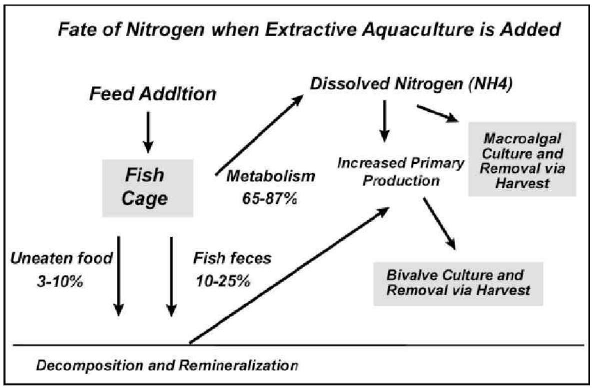

Fig. 2. Pathway of nitrogen wastes in integrated aquaculture systems

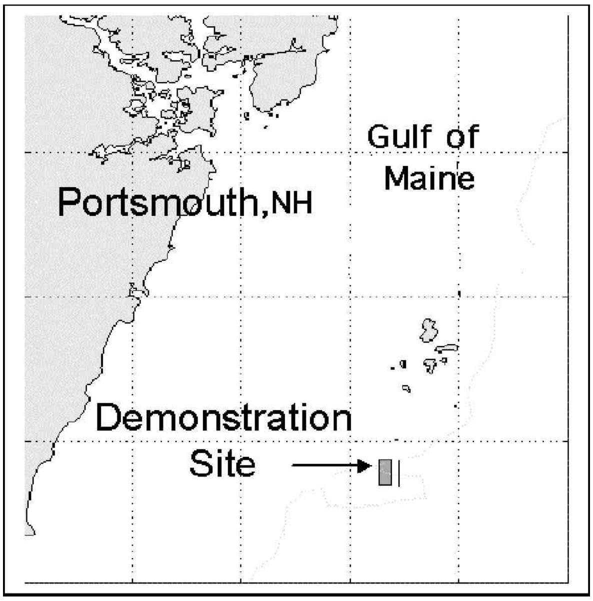

Fig. 3. Map of the New Hampshire and Southern Maine coast showing the location of the open ocean aquaculture demonstration site. The rectangle represents the area in which the fish cages are moored and the adjacent line represents the longlines used for bivalve mollusc culture.

Materials and Methods

Fish and shellfish were cultured in adjacent installations consisting of two submerged sea cages and two submerged longlines. The sea cages were Ocean Spar Technologies, Inc, 600 m^3 Sea Stations, one of which was modified for flatfish culture (Fig. 4). The sea cages occupy a rectangular area of seafloor 24 acres in size, with the length of the rectangle oriented north to south (Fig. 3). The shellfish longlines are located approximately 200 m to the east of the sea cages, and together are 360 m in length. In the current operations, the cages are stocked with 1,500 Atlantic halibut (*Hippoglossoides platessoides*) and 4,000 haddock (*Melanogrammus aeglifinis*) respectively. A proposed expansion of finfish production includes the addition of a third larger cage (3,000 m^3) in 2003 that will be stocked with 37,500 Atlantic cod (*Gadus morhua*). The increased production plan also includes increasing the stocking density of the smaller cages to 25 kg/m^3, which would bring the numbers of halibut and haddock up to 3,750 and 7,500 respectively.

The growout perid for halibut from a 100 gm stocking size to 4 kg is 36 months. Under current stocking density, this would result in total production at harvest of 6,000 kg for the cage. Haddock are grown to 2 kg each in 24 months, yielding a harvest of 8,000 kg.

The amount of nitrogen discharged from finfish operations has been estimated in a number of studies, and most of these have focused on salmon production. The vast majority (87 %) of discharged nitrogen is in the dissolved inorganic form consisting of ammonium (80 %) and nitrate-nitrite (20 %) (Sowles and Churchill, 2002). The estimates range from a low of 30 kg annually per ton of fish, to a high of 78 kg (Sowles and Churchill, 2002). The higher figures were calculated in the early 1990's (Enell and Ackfors, 1991) and the lower discharge estimates in subsequent studies likely reflect improved efficiencies in feed formulation and husbandry practices (Sowles and Churchill, 2002). For this study, an annual value of 50 kg N per ton of fish was used.

The amount of nitrogen removed annually via bivalve harvest was calculated by multiplying the total annual production in meat weight times the percent nitrogen of mussel flesh. At full capacity, each longline can yield 6,000 kg live weight of mussels in a 12 month growout period, resulting in annual production of 12,000 kg whole live weight. Meat yields of mussels cultured at the site have ranged seasonally from 42 % to 60 % with an average of 50 % throughout the year. Estimates of nitrogen content of bivalve flesh range from 1.3 % to 1.8 % (Rice, 2001). A figure of 1.6 % was used in this study.

Since the growout period for halibut is three years, a three-year cycle was used in calculating discharge and extraction. Fish weights and total tonnage from each cage per year was based on observed growth rates for this project (Howell, unpublished data). For halibut, average individual weights of 0.5 kg, 2 kg, and 4 kg were used for years 1, 2 and 3 respectively. For haddock, average annual weights of 0.5 kg and 2 kg were used for years 1 and 2. Year 1 of a second haddock growout cycle was used to calculate the total production weight for year 3. No mortality for either halibut or haddock was assumed in calculating nitrogen discharge. Total weight of mussel meat (and nitrogen removed) was based on observed production and meat yield data from this project (Langan, 2001). Meat yields range throughout the annual growout cycle from a low of 42 % to a high of 60 %. An average meat yield of 50 % was applied to the annual production of 12,000

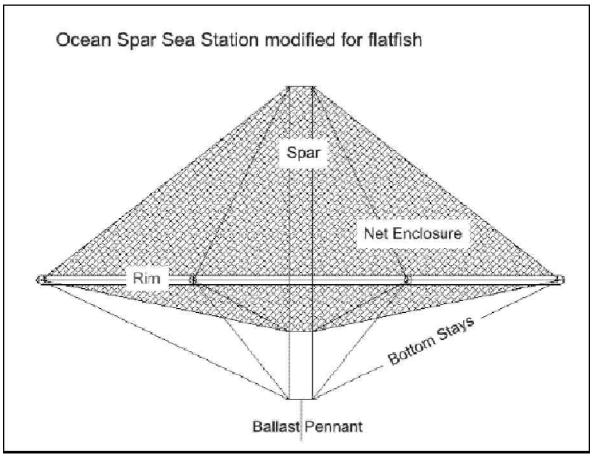

Fig. 4. A schematic of the 600 m^3 OST Sea Station cages used for finfish growout. The cage in this diagram was modified for flatfish culture.

kg whole live weight. Discharge and extraction for the current fish and mussel production (described above) and for the proposed finfish expansion were calculated.

Results

Total estimated nitrogen discharge for the two sea cages is presented in Table 1. For the three-year period, production of 6,000 kg of halibut resulted in a discharge of 488 kg of nitrogen. Haddock production, which was estimated at an 8,000 kg harvest in year 2, followed by a restocking with 4,000 juvenile fish in year 3, resulted in an estimated discharge of 600 kg of nitrogen, bringing the three year total for both species to 1,088 kg of nitrogen.

Nitrogen extraction via mussel harvest as-

Table 1. Calculation of total nitrogen discharge for a three year period for two cages. Wt/fish=the average weight of individual fish at the end of the production year, Biomass=total biomass in the cage at the end of the production year

Species	Year	No.	wt/fish	Biomass	N/ton	N dis
Halibut	1	1500	0.5kg	750kg	50kg	38kg
	2	1500	2.0kg	3,000kg	50kg	150kg
	3	1500	4.0kg	6,000kg	50kg	300kg
Halibut total						488kg
Haddock	1	4000	0.5kg	2,000kg	50kg	100kg
	2	4000	2.0kg	8,000kg	50kg	400kg
	3	4000	0.5kg	2,000kg	50kg	100kg
Haddock total						600kg
Two cage total nitrogen discharge						1,088kg

Table 2. Calculation of the amount of nitrogen extracted via mussel harvest over a three-year period. Total wt=total live weight of mussels and shell, MY=meat yield, MW=meat weight

Year	Total wt	MY	MW	%N	kg N
1	12,000kg	50%	6,000kg	1.6	96kg
2	12,000kg	50%	6,000kg	1.6	96kg
3	12,000kg	50%	6,000kg	1.6	96kg
Total nitrogen extracted					288kg

Table 3. Calculation of total nitrogen discharge for a three year period from three fully stocked cages

Species	Year No.	wt/fish	biomass	N/ton	N dis	
Halibut	1	3750	0.5kg	1,875kg	50kg	94kg
	2	3750	2.0kg	7,500kg	50kg	375kg
	3	3750	4.0kg	15.000kg	50kg	750kg
Halibut total						1,219kg
Haddock	1	7500	0.5kg	3,750kg	50kg	188kg
	2	7500	2.0kg	15,000kg	50kg	750kg
	3	7500	0.5kg	3,750kg	50kg	188kg
Haddock total						1,126kg
Cod	1	37500	0.5kg	18,750kg	50kg	938kg
	2	37500	2.0kg	75,000kg	50kg	3,750kg
	3	37500	0.5kg	18,750kg	50kg	938kg
Cod total						5,626kg
Three cage total nitrogen discharge						7,971kg

sumed three complete growout cycles at the full production capacity of 12,000 kg/year. These calculations are presented in Table 2. For the current operations at the site, which consists of low stocking densities for the two cages, total nitrogen discharge exceeds nitrogen extraction via mussel harvest by 800 kg over a three-year period. Therefore, in order to remove all the nitrogen discharged from finfish production, mussel production would have to be increased by a factor of 2.7, or to 32,400 kg/year. Increasing production to this level would require the installation of six additional longlines.

Similar calculations for nitrogen discharge were applied to the proposed expansion of finfish production and are presented in Table 3. The expansion includes fully stocking the 600 m^3 Sea Station cages to a 25 kg/m^3 density with haddock and halibut, and the addition of a 3,000 m^3 cage with Atlantic cod at the same stocking density. Cod have a two-year growout cycle and annual biomass for year 3 was treated similarly to haddock production in the previous example.

If three cages are fully stocked for growout, the total nitrogen discharged to the environment over a three-year period would be 7,971 kg, or 2,657 kg per year. Based on the calculations presented in Table 2,125 kg of mussels (live weight) need to be harvested to extract 1 kg of nitrogen. Therefore, if a goal for the proposed expansion of finfish production was no net addition of nitrogen, 332,125kg of mussels would need to harvested each year to extract all the nitrogen added by finfish culture.

Discussion

Based on the results of presented here, balancing the total nitrogen discharge from finfish culture operations by integrating extractive species such as bivalves would require the scale of operations for the extractive species to be far larger than the finfish operations. Certainly from an industry perspective, this would create operational management complexity as well as a significant challenge for marketing the large volume of extractive product. The exercise of making these calculations was undertaken to investigate the capacity for mitigating nitrogen addition by culturing an extractive species such as mussels, and is by no means to be construed as advocacy for no net addition of nitrogen for all culture operations. The location of the culture site, the trophic condition and assimilative capacity of the receiving waters, and marketing landscape must all be taken into consideration in order to determine the most effective approach to integrated aquaculture. In locations such as the Gulf of Maine where this project is being conducted, the coastal waters are naturally high in nitrogen due to upwelling of deep nutrient rich waters. It is highly unlikely that operations in offshore environments with high ambient nitrogen concentrations and large dilution capacity would be impacted by discharge from finfish culture. Sowles and Churchill (2002) investigated the potential exposure and effect of nutrient enrichment by salmon culture in Cobscook Bay, Maine, U.S.A. They found that despite the significant loading associated with annual production of 6,000,000 kg of salmon, there was little change in water column nitrogen concentration and no discernable increase in primary production. Similarly, Wildish *et al.* (1992) reported difficulty measuring differences in nitrogen concentration from ambient conditions beyond a few meters from the cages in an intensely farmed area of the Bay of Fundy. Whether or not nitrogen plumes above ambient conditions can be measured should not be the only criteria for determining whether finfish operations should be permitted, or mitigation by extractive species should be required. Determining the assimilative capacity and nutrient sensitivity of the surrounding waters is extremely important (Chopin *et al.*, 2001). In some cases, an increase in nutrient loading may result in beneficial increases in secondary production without any associated negative impacts. Nixon and Buckley (2002) examined nutrient loading in relation to fisheries growth and biomass data from the Baltic Sea. They found that fish growth and biomass were significantly greater with increased nitrogen loading in the latter throughout the 20th century. Concerns over nutrient additions to coastal waters are in many cases valid, however, it should be recognized that nutrients are essential for producing biomass, and may potentially result in greater fisheries yields. Certainly, if aquaculture operations are to be sited in waters that are already eutrophic or have been scientifically determined to be nitrogen sensitive, they should be of the extractive type or at minimum strive to balance inputs with removal.

As illustrated by the exercise undertaken in this study, if no net increase in nitrogen is a goal for aquaculture development, it would be difficult to achieve without shifting the emphasis from finfish to extractive species such as bivalves and seaweeds, or by vastly reducing discharge by improving feed formulations and nitrogen assimilation by the fish. Nutrient issues aside, there may be other reasons for a more integrated approach to aquaculture development. Among these are to counter the negative perception of aquaculture by the environmental and regulatory communities, to increase profit margins with revenue from secondary crops and to reduce the financial risk of relying on a single species.

Acknowledgements

The research presented in this paper was made possible by a grant from the U.S. Department of Commerce, National Oceanic and Atmospheric Administration to the University of New Hampshire's Cooperative Institute for New England Mariculture and Fisheries (CINEMAR).

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水研センター研報，別冊第1号，65−69，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 65-69, 2004

Environmental carrying capacity in an aquaculture ground of seaweeds and shellfish in Sanriku coast

Ken FURUYA*

Abstract In non-feeding aquaculture of seaweeds and shellfish the culture organisms compete with natural populations for resources, viz. nutrient salts and food particles. Therefore evaluation of carrying capacity of coastal waters is crucial for sustainable exploitation of biological productivity. For this two major criteria are proposed: accurate estimation of phytoplankton primary production which governs the magnitude of total biological productivity, and understanding of oxygen dynamics based on a preliminary study in a bay on the northeastern coast of Japan.

Key words: carrying capacity, shellfish culture, seaweed culture, Sanriku coast

Non-feeding aquaculture of seaweeds and shellfish recovers nutrient loadings from the land. Since there is no other industrial way of recovery, this function is very important in controlling nutrient cycling between land and coastal waters. The amount of the recovery by short-neck clam fisheries is estimated to be about a sixth of the total input of nitrate in Hamana Bay in central Japan (A. Hino, personal communication). Knowledge of material cycling in the natural ecosystem is prerequisite to utilize coastal productivity in a sustainable manner, as the cultured organisms are dependent on the natural ecosystem. Nutrients, the key factor that fuels primary production, are supplied by advection, riverine input and regeneration, and utilized by phytoplankton and macroalgae competitively. Then, organic matter produced by phytoplankton, naturally occurring and cultured macroalgae is consumed not only by zooplankton and benthic organisms, but also by cultured scallops and oysters. Thus it is important to quantify the production at different trophic levels for evaluation of the carrying capacity of coastal waters.

Tatara (1992) partitioned the distribution of organic matter produced by primary producers to heterotrophs and traced a grazing food chain. In this work, several factors were considered that determine the carrying capacity of coastal waters for fisheries production. One of the major hurdles recognized was the poor availability of biological data. In coastal areas, primary production of phytoplankton markedly fluctuates in the time scales of hours to weeks. Although the bottle incubation technique has been traditional and standard in the measurement of primary production, its spatio-temporal coverage is strictly limiting.

A study on material cycling in an aquaculture ground for seaweeds and shellfish is in progress in Otsuchi Bay. The bay located at 39° 20'N, 141° 56'E is 8 km long and 2 km wide, and opens into the western North Pacific Ocean. Owing to the freshwater flow into the innermost part, and the long and narrow topography of the bay, exchange of water in the bay is expected to be brought about as, a seaward outflow of the surface water over landward-moving inflow of denser, more saline subsurface water from outside the bay (Shikama, 1990). This circulation occurs fre-

2003年6月12日受理（Received on June 12, 2003）
* Graduate School of Agricultural and Life Sciences, The University of Tokyo Bunkyo, Tokyo 113-8657, Japan

quently during winter and spring when the westerly seasonal wind prevails. Conversely, an inflow of surface water over outflow of deeper water is also observed during the summer. This circulation pattern may alter from the former to the latter, and vice versa in summer, depending on the difference in water density between inside and outside the bay. The formation of spring bloom depends on the wind-driven circulation. The outward flow of the surface water interrupts formation of the spring bloom, and transports phytoplankton population seaward (Fig. 1, Furuya *et al.*, 1993). By such water movements, a significant amount of nutrients in the bay is brought out, or replenished into the bay in the subsurface layer, depending on water masses located outside the bay. Therefore, the water movement governs in- and outward flux of materials in the bay. Owing to this physical condition, high temporal resolution of observation is of particular importance in the study of material cycling in the bay.

Preliminary results of the study are summarized in Furuya (2004) and briefly outlined here.

Primary production and dissolved oxygen on the bottom

A bio-optical approach was used for rapid monitoring of chlorophyll a and primary production with high temporal resolution. The

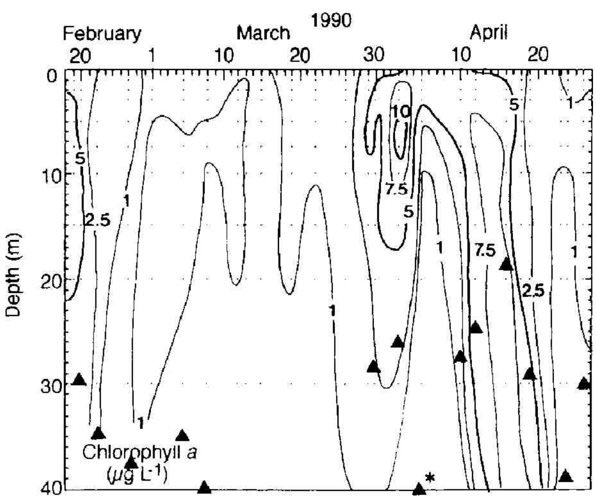

Fig. 1. Chlorophyll *a* at a station in Otsuchi Bay during the spring of 1990 (Furuya *et al.*, 1993)

natural fluorescence technique (Chamberlin and Marra, 1992) was improved for use in coastal waters (Yoshikawa and Furuya, in press) and applied for estimating chlorophyll a and phytoplankton primary production in the bay. Both calculated chlorophyll a and primary productivity from natural fluorescence showed significant correlations with the directly measured values during four seasons.

The integrated primary production throughout the water column was estimated from data obtained by a moored fluorometer, and extrapolated to the production of the whole bay. The primary production of the entire bay was estimated to be 1.7×10^3 ton C during a period from mid January to late April.

The cultured kelp *Undaria. pinnatifida* (locally called wakame) showed steady growth until March, while growth was much reduced in April (Yoshikawa *et al.*, 2001). Maximum biomass of 840 ton in wet weight (28.3 ton C, and 2.54 ton N) was recorded in early March, just prior to the harvest, and these figures decreased as the harvest continued. During April a major portion of the primary production was lost by removal of aged part of the thallus. Production of cultured *U. pinnatifida* throughout Otsuchi Bay increased steadily in January and February, and reaching maximums of 1055 kg C d^{-1} and 99.0 kg N d^{-1} in March. Erosion of the alga began in early March, and peaked at a rate of 469 kg C d^{-1} and 43.1 kg N d^{-1} in mid March. Erosion declined gradually as harvesting continued, and was comparable to production rates during the month of April.

The biomass harvested was 38.7 ton C (81 % of total) while that lost due to erosion was 10.8 ton C corresponding to 19 % of the total biomass produced.

Phytoplankton primary production consistently exceeded that of *U. pinnatifida* by more than 20 times and was 38 times higher on average (Fig. 2). Variations in phytoplankton primary production was ascribed primarily to the change in phytoplankton biomass, which was largely controlled by water circulation (Furuya *et al.*, 1993).

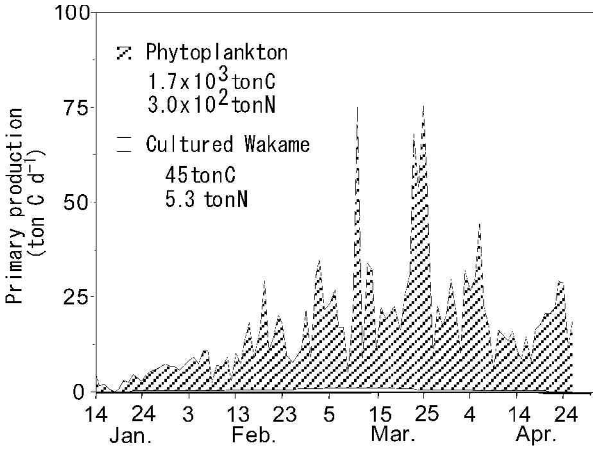

Fig. 2. Primary production of phytoplankton as measured by natural fluorescence and that of wakame (Furuya, 2003)

Organic matter loadings and dissolved oxygen concentration in bottom waters were examined near the shellfish culture areas under culture rafts of a scallop *Patinopecten yessoensis* and the Japanese oyster *Crassostrea gigas*. Sinking flux of organic matter, mainly composed of fecal matter was significantly higher under the culture rafts of the oyster than under those of the scallop. The flux under the oyster rafts was 21.6 mg C m^{-2} d^{-1} on average in spring and summer, while that under the scallop rafts was 7.75 mg C m^{-2} d^{-1}. The mean flux outside the raft area as control was 5.75 mg C m^{-2} d^{-1}. Oxygen consumption rate of bottom seawater taken under the scallop rafts as determined by dark bottle incubation ranged from 0.26 to 3.07 mg L^{-1} d^{-1} with a mean of 1.49 mg L^{-1} d^{-1}. The mean *in vitro* rate implied rapid depletion of dissolved oxygen near the bottom in 5 days. However, *in situ* dissolved oxygen was never exhausted in summer varying between 4.34 and 7.19 mg L^{-1} d^{-1}. *In situ* continuous monitoring showed steady but slow decrease in dissolved oxygen during the summer at a mean rate of 0.041 mg L^{-1} d^{-1}. This apparent rate indicates that it should have taken 160 days to produce anoxic water. These observations show continuous supply of dissolved oxygen through water flow along the bottom is large and maintain the favorable oxygen field near the bottom in summer. Wind-induced circulation, density current and, in particular, internal tide are responsible for the inflow of the outside water along the bottom (H. Otobe, in preparation).

In Sanriku coast there is an array of bays with similar topography to that of Otsuchi Bay. Therefore, an active inflow of subsurface water can be expected from outside which prevents depletion of dissolved oxygen. On the contrary, anoxic water mass is formed near the bottom of Ofunato Bay which located in the southern part of the Sanriku coast. There is a sill in the mouth part of the bay that reduces water exchange considerably, being responsible the formation of anoxic water together with the intensive culture of the Japanese oyster. With comparison of the water circulation and associated oxygen field near the bottom sediment between Otsuchi Bay and Ofunato Bay, it is clear that dissolved oxygen is important for sustainable exploitation of biological productivity in aquaculture areas. Other bays on the Sanriku coast, water exchange is likely active according to bottom topography. Therefore, the Sanriku area is in general suitable for aquacultures in a point of view of oxygen supply.

Shellfish culture

Cultures of scallop and oyster occupy more than 95 % of total annual production of shellfish in Japan (Fig. 3). The annual production has been rather constant over recent ten years.

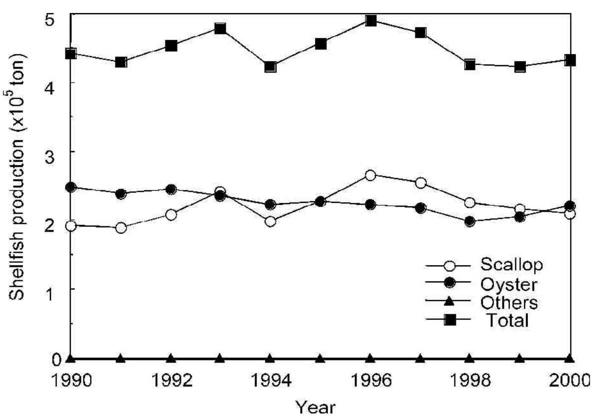

Fig. 3. Annual production of shellfish culture in Japan (Ministry of Agriculture, Forestry and Fisheries Japan, 2001)

Almost all of the production is consumed as foods for human with a small portion for pet foods. Among the major reasons for the constant production over years, saturation of market and maturation of culturing technique are considered to be most responsible (H. Kurokura, personal communication). Considering the significance of shellfish culture as recovering means of nutrient loadings, utilization of unexploited potentials of shellfish culture should be challenged intensively. For development of the market, one possibility is use of shellfish as materials of forage production. Since there is increasing demand of foods of livestock and fish culture in Japan, self-support ratio of food is under the influence in Japan. Potential importance of shellfish as protein source may be acknowledged by selection of suitable species for culture, and technical development of food processing for forage production. Nevertheless, there should be various uncertainties how compatible a new species is introduced with current culture of scallop and oyster. Therefore, optimized utilization of biological production is emphasized for the introduction of new species as well as sustainable development of scallop and oyster culture.

Conclusion

The observation during the study serves development of numerical physical-biological coupled models (Kawamiya *et al.*, 1995; Kishi *et al.*, 2003; Kishi *et al.*, in prep.). Kishi *et al.* (2003) incorporates the aquaculture of both wakame and shellfish into the model. The continuous monitoring of flow field, chlorophyll a, primary production and dissolved oxygen with high temporal resolution serve robust validation of the model performance and improvement of the model. Evaluation of carrying capacity of the bay is in progress using the model to understand the importance of phytoplankton primary production and dissolved oxygen and to identify other key factors. The model is intensively used to optimize aquaculture production of seaweeds and shellfish with least environmental impacts in the bay.

Acknowledgements

I thank M. J. Kishi, H. Otobe, T. Yoshikawa, M. Takagi, S. Kaga and K. Sekiguchi for their cooperation throughout the study.

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水研センター研報，別冊第1号，71−76，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 71-76, 2004

Review: Production of *Gracilaria parvispora* in two-phase polyculture systems in relation to nutrient requirements and uptake

Erin RYDER*[1] , Stephen NELSON*[2], Edward GLENN*[2], Pamela NAGLER*[2], Sherman NAPOLEAN*[3] and Kevin FITZSIMMONS*[3]

Abstract *Gracilaria parvispora* Abbott is highly valued in Hawaiian seafood markets. Due to the over-harvesting of natural beds. *G. parvispora* is scarce on the open reef ; and harvesting is strictly regulated. On Molokai, Hawaii, a community-based operation was established to develop a sustainable, integrated culture system for this species. Previous research suggested that ammonium was the limiting factor for sufficient growth on the reef. Therefore, on Molokai, a polyculture system was developed using fish/shrimp effluent to load thalli with nitrogen before placement in a low-nutrient lagoon for grow-out. The research described here demonstrates how small-scale, commercial culture of seaweed can be successfully integrated with the production of fish and shrimp. Two benefits of a two-phase polyculture system are: 1) a waste product from the first phase (i.e. ammonia nitrogen) becomes a resource for the second phase and 2) integrated systems are financially more stable because of improved cash-flow and product diversification. A modest biomass of fish can support a substantial production of seaweed. The type of cage-based, polyculture system developed on Molokai could be applicable to other rural coastal areas.

Key words: *Gracilaria parvispora*, polyculture, tank culture, effluent, nitrogen

Introduction

Intensive tank and pond culture of free-floating seaweeds has been developed over the last two decades in Israel (Friedlander and Levy, 1995), Taiwan, Chile, the United States, Canada (Buschmann, 2001) and other areas. Advantages of these methods include a high potential yield, the possibility to control and mechanize operations and the possibility of using seawater ponds as biological filters for fish culture and other effluents (Friedlander and Levy, 1995). A disadvantage is the high cost compared to less intensive culture methods. However, on the island of Molokai, Hawaii, we have developed a two-phase polyculture system for production of seaweeds that has the advantages of more intensive culture systems but can be operated at low cost.

The integrated aquaculture system described here was designed as a model for sustainable, small-scale crop production in rural areas of the tropical Pacific. The research was initiated partly in response to declines in local seaweed resources in Hawaii, where seaweeds such as *Gracilaria parvispora* are valued, traditional foods. In Hawaii, over-harvesting of *G. parvispora* from the fringing reefs started in the 1970s, and this led to a severe decline in natural stocks. As a result, conservation laws

2003年6月12日受理（Received on June 12, 2003）
*[1] University of Maryland, Center for Environmental Science, P.O.Box 775, Cambridge, MD 21613 USA Email: Erin_Ryder@hotmail.com
*[2] University of Arizona Dept. of Soil, Water, and Environmental Science, 2601 East Airport Drive, Tucson, AZ 85706, USA
*[3] Ke Kua'aina Hanauna Hou, HC01 Box 741, Molokai, HI 96748-0741, USA

have been enacted that limit wild-crop harvesting; and commercial harvesters cannot take more than 4 kg per day. In addition, it is forbidden to remove cystocarpic thalli from the water. The initial restoration project, whose purpose is to develop a cultivation system to re-establish *Gracilaria* on the reef, was instituted as a result of declining wild stocks.

The project was developed in conjunction with a non-profit, community-development organization on Molokai. The group, Ke Kua'aina Hanauna Hou (KKHH), was established in 1991 to develop ways to help the local community by developing means for individuals or groups to earn an income while maintaining the local land-use traditions. Although they participate in the modern economy, many Molokai families still derive a substantial percent of their livelihoods from fishing, gardening, and other subsistence activities that help them maintain a connection with their traditional value system, including a sense of stewardship towards their island's natural resources. Marketing seaweeds is one way for native Hawaiians to achieve this.

In Hawaiian seafood markets, an edible, red seaweed *G. parvispora* known locally as "long ogo" is highly valued (Glenn *et al.*, 1998). Total sales of *G. parvispora* by KKHH in 1999 were 10,670kg with a wholesale market value of approximately $75,000. By combining ogo culture with shrimp and fish culture, production has increased substantially. Molokai long ogo sells for $7 per kg (a $2.20 per kg premium over other seaweeds on the market), and sales of Molokai ogo was approximately $250,000 over the last 12 months.

In previous work, Glenn *et al.* (1998, 1999) demonstrated that *G. parvispora* is limited by nitrogen on the south reef of Molokai, HI. This led to the development of a labor-intensive culture system where the seaweeds were produced in cages in the nutrient-poor coastal area and then brought in each week to be fertilized in tanks. More recently, the production system was modified to reduce labor and increase yields by integrating the cage grow-out system with the local production of fish and shrimp, which are examples of other small-scale commercial aquaculture enterprise that have developed on Molokai.

The environmental benefits of integrating the production of aquatic plants with the production of fish or shrimp to recapture nutrients are well known (e.g., Buschmann *et al.*, 1994; Neori *et al.*, 1996; Shpigel and Neori, 1996; Chopin and Yarish, 1998; Mathias *et al.*, 1998; Troell *et al.*, 1997, Petrell *et al.*, 1992.). Most of the research on integrated plant-animal aquaculture systems has involved intensive, land-based culture methods (Buschmann, *et al.*, 1994; Neori *et al.*, 1996; Shpigel and Neori, 1996). Growing multiple cash crops such as shrimp, fish, bivalves, and seaweeds in extensive systems is common in Asian aquaculture systems (Chen, 1990), but this has not been widely adopted in western aquaculture.

The focus of this paper is to describe the application of this concept to the development of a two-phase polyculture system that has been successful on Molokai and could therefore serve as a model for similar small-scale commercial development in other tropical areas.

The Molokai two-phase polyculture system

Since 1992, the University of Arizona together with Ke Kua'aina Hanauna Hou worked at developing and refining a community-based system for culturing *Gracilaria*. The system now incorporates: a hatchery (Nelson and Glenn, 2000), which produces spore-coated substrates for distribution to outside growers; outplants of sporelings on the reef or in polyculture with fish and shrimp; a cage-culture farm for multiplication of the harvest; and a processing facility. The two-phases of seaweed culture in this system are an enrichment phase and a grow-out phase (Fig. 1). The overall method involves all the life stages of the seaweed crop and enhances wild stocks on the reef. The system incorporates the major strategies of ecological design: conservation, regeneration, and stewardship (Van der Ryn and Cowan, 1996).

From research in outplanting of spore-laden materials it was found that the success of sporeling development was related to dissolved ammonia levels in the immediate vicinity (Glenn *et al*, 1999). This led us to look at growing the seaweeds in conjunction with fish or shrimp, since the primary excretory product of aquatic animals is dissolved ammonia. Our first trials involved marine shrimp culture, because one of the local shrimp farms was found to have dense populations of *G. parvispora* growing in its effluent ditch. We transferred nutrient-depleted, tagged thalli to the ditch and monitored their growth and nutrient content along with levels in the effluent (Nelson *et al.*, 2001). Because of the periodic draining of shrimp ponds into the ditch, nutrient levels fluctuated widely from day to day, but mean ammonia levels in the ditch (approximately 62 mmol m^{-3}) were many times higher than in nearby coastal waters. In this environment the thalli nitrogen content was increased from approximately 1 % to 3 % within about 5 days. We also found that when these thalli were placed in the lagoon for grow-out they grew at rates of 8 % to 10% per day for the first week. Growth and thalli nitrogen content declined over the 4-week growth period so that by the fourth week, growth was approximately 2 % per day and the nitrogen content was back to 1 %. Growth rates of thalli that were enriched in the ditch are shown in Fig. 2. This method of enriching the thalli requires much less labor than the weekly use of chemical fertilizers in tanks as was previously practiced.

Seaweed produced in the shrimp effluent channel became an important source of new material for stocking grow-out cages in the lagoon. Harvested seaweed from the ditch was not directly marketable because of heavy loads of sediment and epiphytes. However, after a period of grow-out in which seaweed biomass was increased in clean water, the product was of high quality and easily sold in local markets.

Success with the use of shrimp effluent led us to establish a tank culture system for production of local fishes at the seaweed production site (Nagler *et al.*, 2003). This allows more control of the production cycle and further decreases labor, by eliminating the need to

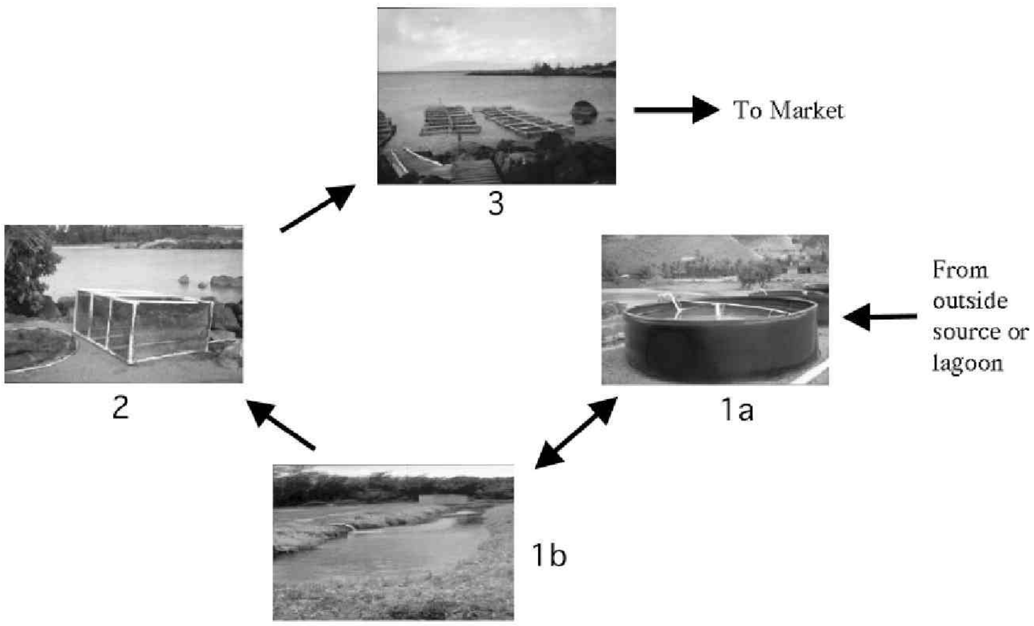

Fig. 1. Schematic of Ke Kua'aina Hanauna Hou's polyculture system for production of *Gracilaria* parvispora. Seaweed is supplied by outside growers or hatchery then fertilized in shrimp/fish effluent channels (1a, 1b). After enrichment with nitrogen, the seaweed is placed in floating cages (2) and floated in Puko'o lagoon for growout (3). After 10 days in the lagoon, the seaweed is cleaned and sold to market.

harvest seaweed from the effluent ditch and transport of the harvest to the grow-out facilities. Milkfish (*Chanos chanos*) and mullet (*Mugil cephalus*) juveniles were obtained from the Oceanic Institute on Oahu and were cultured in tanks onshore immediately adjacent to the seaweed grow-area. Milkfish grew much better in the tanks than mullet and is now the primary species being cultured.

Nagler *et al.* (2003) were able to densely stock the fish tanks with nutrient-poor seaweed for periods of 7 days in order to enrich the thalli. Over the enrichment period the thallus nitrogen content increased up to 5 % on a dry weight basis. The growth rate of enriched thalli was up to 10 % per day and production in the cages ranged from 39-57 g dry mass per m^2 per day over a 21-day production cycle. In these trials with fish, we estimated the nitrogen requirements of *Gracilaria* and calculated the amounts of fish feed needed to provide these amounts, without calculating the actual excretion rates of the fish. Subsequently, however, in order to provide data useful in designing a similar integrated farm, we measured the excretion rates of fed milkfish as the amount of nitrogen excreted per gram of fish.

For the excretion studies (unpublished), milkfish of various sizes were held individually in 55-L, aerated tanks that were filled with seawater from the grow-out site on Molokai. Water samples from the tanks were taken immediately after the fish were added and at 2-hour intervals thereafter. We used a fluorometric method for determining the concentrations of dissolved ammonia (Holmes *et al.*, 1999). Data for the first 2-hour period are shown in Fig. 3, which shows the relationship between fish mass and the ammonia excretion rate in a log-log plot. Mean excretion rates were 7.03, 2.17, and 1.81 μg g^{-1} per hour for mean fish weights of 200 g, 800 g, and 1200 grams, respectively.

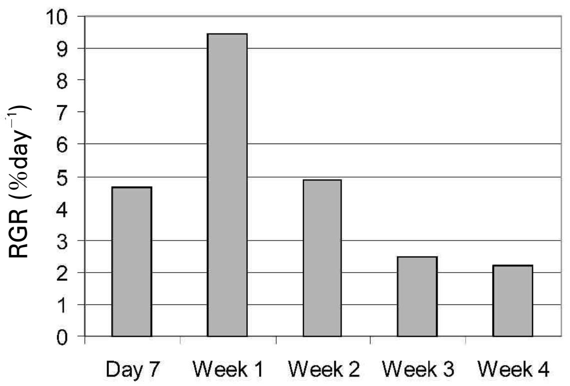

Fig. 2. *Gracilaria parvispora* relative growth rates (RGR) after enrichment in a shrimp effluent channel. Day 7 growth rates occurred during the enrichment process. Week 1 depicts growth after placement in lagoon. Seaweed experienced initial rapid growth during the first week, then lower growth on subsequent days. Data are from Nelson *et al.* (2001).

Projections

Data from these studies was used to calculate the fish biomass required to support the seaweed production levels usually achieved at the Molokai grow-out site. This is a small-scale cage culture operation that typically has about 80 cages in production throughout most of the year. The optimal stocking density for the cages, in terms of the percent increase of seaweed biomass, was determined to be approximately 2 kg per cage (Nagler *et al.*, 2003). Table 1 shows the requirements of the biomass and number of fish needed to support a variety of seaweed production projections. As shown, even at high stocking densities, the amount of fish needed to support *G. parvispora* growth is relatively small. Considering KKHH is a small,

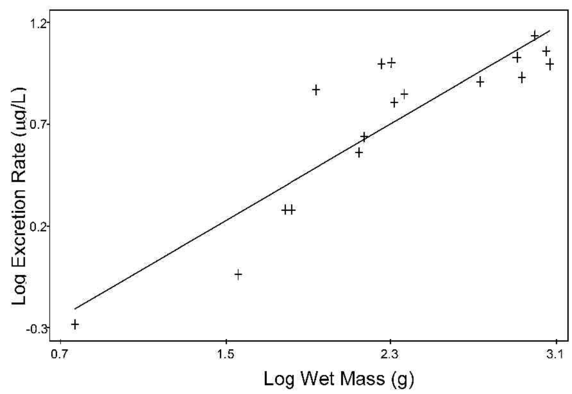

Fig. 3. Relation between wet fish biomass and excretion of ammonium (μg/L).

Table 1. Number and biomass of milkfish required to support (to increase nitrogen content of thalli from 1 to 4 %) the production of Gracilaria parvispora in a two-phase polyculture system in Hawaii

Total algal biomass	0.2-kg fish		0.8-kg fish		1.2-kg fish	
(kg wet weight)	kg fish	# fish	kg fish	# fish	kg fish	# fish
40	17	85	55	70	7	57
80	34	170	110	140	133	115
160	68	340	220	275	265	230
320	140	680	440	553	530	460
640	270	1,365	885	1,106	1,060	917

community-based operation, they can sufficiently support their ogo production using fish effluent as a nutrient source. Their current operation consists of several tanks containing enough milkfish to enrich the seaweed that is in the production system.

Conclusions

The production of *Gracilaria* in cage culture can be successfully integrated with small-scale production of fish and shrimp. Seaweed stocked in shrimp culture effluent ditches can thrive with little management and can serve as stocks for grow-out in clean, low-nutrient water. We were able to achieve similar enrichment of *Gracilaria* thalli by holding stocks for 5 to 7 days in fish tanks. We found that a even a modest biomass of fish can support a substantial production of seaweed in this culture system. This was also the case in studies of *Gracilaria* cultivated with salmon grown in cages (Troell *et al.*, 1997; Buschmann *et al.*, 2001), where increased productivity of 1 hectare of *Gracilaria* could be supported with 5 % of the fish dissolved nitrogenous waste. In the culture operation on Molokai the main benefits of nutrients are to support seaweed production rather than to scrub nutrients for effluent. However, if seaweeds are cultured on a larger scale, such as for the phycocolloid industry, they can be efficient at removal of nutrients from effluent of intensive land-based fish farms (Troell *et al.*, 1997; Chopin *et al*, 2001).

Disregarding the environmental benefits of integrated seaweed and fish or shrimp production, seaweed culture can also benefit small-scale aquaculture farms by increasing their economic viability. Small aquaculture enterprises for shrimp or fish, such as those on Molokai, tend to be financially unstable, because they have continuous, fixed expenses but intermittent income because their harvests come in batches that are spaced weeks or months apart. Integration of seaweed production with these systems greatly improves cash-flow, as seaweeds can be harvested weekly throughout the year. The seaweed component in our system is worth approximately $7 per kg and revenues from seaweed sales could significantly enhance the profitability of an integrated system. On Molokai, the use of fish or shrimp effluent increased production of *Gracilaria* and also resulted in reduced labor requirements, an additional economic benefit. In addition, diversification of farms in the community can aid in reducing competition and stimulate the formation of cooperative relationships among producers. This type of cage-based, polyculture system developed on Molokai could also be successfully employed in other rural areas of the Pacific.

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Preliminary examination of the bioremediation and mariculture potential of a Northeast U.S.A. and an Asian species of *Porphyra*

George P. KRAEMER*[1], Raquel CARMONA*[2,3], Christopher NEEFUS*[4], Thierry CHOPIN*[5], Sheryl MILLER*[3], Xiaogeng TANG*[3,6] and Charles YARISH*[3]

Abstract Finfish and shrimp mariculture operations produce nutrient-rich effluent that can threaten the health of coastal ecosystems if not properly managed. As part of an effort to develop an economically viable system of integrated polyculture, we have begun to evaluate the bioremediation and mariculture potential of Northeast U.S.A. and Asian species of *Porphyra*. We present here preliminary results based on short- and long-term experiments. Short-term nitrogen (N) uptake measurements were conducted over ca. 20 min in 50 mL tubes at 5-15 ℃ and at high (10 g FW L^{-1}) stocking density. During long-term (28-d) experiments at 15 ℃ and at 0.4 g FW L^{-1}, we examined the growth, N assimilation into *Porphyra* tissue, and phycobiliprotein contents at three- to seven-day intervals as a function of N concentration (25, 75, 150, 300 μM). Performance (growth rate and bioremediation) was maximal at 150-300 μM inorganic N. Induction of archaeospore production reduced growth rates. *Porphyra purpurea* removed 96-100 % of N within 3.5 days at 150 μM NH_4^+. Overall, *Porphyra* appears to be an excellent choice for bioremediation of moderately eutrophic effluents, with the added benefit that tissue may be harvested for sale.

Key words: *Porphyra*, eutrophication, aquaculture, mariculture, nitrogen, phosphorus, bioremediation, seaweed

Introduction

Finfish and shrimp mariculture produces effluent that is rich in inorganic nitrogen (N) and phosphorus (P). These nutrients derive from the bacterial release of inorganic N and P from non-consumed animal food, and from excretory waste products of the cultured animals (Beveridge, 1987). These additions of N and P can threaten ecosystem health by degrading critical functions of coastal ecosystems (McVey *et al.*, 2002). The detrimental effects of eutrophication include blooms of harmful phytoplankton and unwanted macroalgae (Cuomo *et al.*, 1993), as well as development of hypoxia and anoxia (Sfriso *et al.*, 1987). The ecological incentive for remediating eutrophic effluents is clear. Moreover, there are also economic incentives to reduce the poss of nutrients. The N and P that are flushed from the system represent a loss of opportunity for the aquaculturist, since these nutrients could be channeled into the production of valuable products. In addition, governmental agencies charged with reducing coastal eutrophication are developing regulations limiting the release

2003年６月12日受理（Received on June 12, 2003）
*[1] Department of Environmental Sciences, State University of New York, Purchase College, Purchase, NY 10577 USA
*[2] Department of Ecology and Evolutionary Biology, University of Connecticut, Stamford, CT 06901 USA
*[3] Faculdade de Ciências do Mar e do Ambiente, Universidade do Algarve, PORTUGAL
*[4] Department of Plant Biology, University of New Hampshire, University of New Hampshire, Durham, NH 03824
*[5] Centre for Coastal Studies and Aquaculture, University of New Brunswick, Saint John, New Brunswick E2L 4L5 CANADA
*[6] College of Marine Life Sciences, Ocean University of Qingdao, PRC

of N and P (c.f. U.S.A. Environmental Protection Agency Proposed Effluent Guidelines Program Plan, Federal Register (June 18, 2002, Volume 67, Number 117); Jordan, 2002). In the future, aquaculturists can expect to incur financial penalties if they cannot regulate the release of their waste effluent.

One solution to the problem of eutrophic effluent that addresses both ecological and economic issues is the development of integrated aquaculture, in which seaweeds are grown downstream from animals. Systems of integrated aquaculture are ideal because the N and P in the animal effluent are necessary requirements for the growth of the seaweeds. This is not a new idea (for review see Chopin *et al.*, 2001). What distinguishes this project from many of the prior studies is the selection of the algal component. Obviously, the best seaweed to integrate into an animal aquaculture operation is one characterized by rapid growth, the accumulation of N and P to high levels in tissue, and some added value. We are investigating the genus *Porphyra* as the seaweed partner for a number of reasons (Yarish *et al.*, 1998; Chopin *et al.*, 1999). All species of *Porphyra* produce gametophytes that are flat sheets one or two cell layers thick. This combination ensures an extremely high surface area-to-volume ratio, with all cells involved in the uptake of nutrients and production of new tissue. In part for these reasons, *Porphyra* is capable of rapid growth; preliminary measurements show this genus capable of at least 20 % d^{-1} (Kraemer, pers. obs.). Species of *Porphyra* also are efficient nutrient concentrators. In situations where nutrients are readily available, N can constitute up to 7.4 % of the dry tissue biomass (Chopin and Yarish, 1998, 1999). Finally, several species of *Porphyra* form the basis for a multi-billion dollar (U.S.) global business in the production of nori for human consumption. A recent added value under development is the use of R-phycoerythrin as the fluorescent conjugate for immunological dectection of target molecules in molecular biological research (Mumford and Miura, 1988).

Previous studies have focused on the integration of other macroalgal species into finfish culture (e.g., *Ulva lactuca*; Cohen and Neori, 1991). While other species may be efficient nutrient filters, the biomass that is produced has limited application after harvest (e.g., organic composting). Consequently, *Porphyra* not only has potential for bioremediation of aquaculture effluents, but also production of a crop that may have economic value.

Growth, accumulation of N and P, and high value by-products in tissue are dependent on the environmental factors that regulate production: temperature, nutrient availability, irradiance, water motion. As part of an effort to develop an economically viable system of integrated aquaculture, we have been evaluating the bioremediation and mariculture potential of Northeast U.S.A. and Asian species of *Porphyra*. We have included Asian species in the comparison since they represent in many ways the industrial benchmarks. However, we do not advocate the use of non-native species of *Porphyra* in open culture in New England coastal waters. We present here preliminary results that describe the influence of temperature and nutrient availability on N uptake, tissue production, phycoerythrin and N tissue content, and nutrient removal efficiency.

Materials and Methods

We have been working with aquaculturists from GreatBay Aquaculture, LLC. (GBA; Portsmouth, New Hampshire, U.S.A.) in the development of the integrated system. GBA raises summer flounder (*Paralichthys dentatus*) and cod (*Gadus morhua*) in a land-based system. Effluent from the grow-out tanks has an average NH_4 concentration of 150 μM, and temperatures that range from 10 to 19°C over the course of the year. These values were used to determine the conditions for the laboratory experiments.

All blade (gametophyte) tissue used for these experiments was generated from the conchocelis stage. The experimental work was conducted under controlled environmental conditions of irradiance, temperature, and photoperiod in walk-in growth chambers at the University of Connecticut, Stamford. We present here data from the Northeast U.S.A. species *P. purpurea* (New York strain for long-term experiment and Maine strain for short-term N uptake measurements) and *P. yezoensis* (an Asian cultivar). Short-term measurements of NH_4^+ uptake rate were performed on tissue grown at 5 , 10 , or 15 ℃. Samples (ca. 0.3 g FW) were placed in 50 mL transparent plastic screw-top tubes containing 30 mL of artificial seawater ("MBL" formula, Cavanaugh 1975). Ammonium levels were adjusted to 10, 20, 40, 75, or 150 μM while inorganic phosphorus was added to a constant N:P molar ratio of 10:1. Tissue was incubated at the growth temperature. At 7 and 17min after the introduction of tissue into the tubes, samples of incubation medium were collected and analyzed for NH_4^+ concentration (Liddicoat *et al.*, 1976). Five to seven replicates were used per N concentration.

Long-term (28-d) experiments were also carried out at the University of Connecticut facility in 1-L flasks. *Porphyra* gametophytic tissue (ca. 0.4 g FW L^{-1}), grown at 10 ℃ and acclimated at 15 ℃ for seven days, was placed in 800 mL of von Stosch-enriched culture medium. The N level in the incubation medium was adjusted to either 25, 75, 150, or 300 μM, with P added to constant 10:1 N:P molar ratio. Three replicates at each N concentration were used. At 3-4 d intervals the culture medium was changed, with a sample from each flask retained for N and P analysis (via Technicon autoanalyzer). At 7-d intervals, the tissue in each flask was weighed (FW). Newly produced tissue was removed so that each 7-d growth period began with the same stocking density (0.4 g FW L^{-1}). From the excised tissue, samples were conserved for CHN (by means of a Perkin-Elmer CNS analyzer) and phycobiliprotein analyses (Beer and Eshel, 1985).

Results

Porphyra purpurea demonstrated differences in the rate of NH_4^+ uptake that were a function of temperature (Fig. 1). The highest rates of NH_4^+ uptake by P. purpurea occurred at 10 ℃. *Porphyra yezoensis* took up NH_4^+ roughly twice as fast at 15 ℃ than did *P. purpurea*.

Over the 28-d long-term experiments, growth was not constant for either species (Fig. 2). *Porphyra yezoensis* grew well during the first week (18 % d^{-1}), but archaeospore production ensued and the tissue began to disintegrate. By the experiment's end, the *P. yezoensis* growth rate was negative as losses due to reproduction exceeded new growth. *Porphyra* purpurea appeared to enter a brief period of archaeospore production but growth rebounded by the experiment's end.

By the experiment's end, phycobiliprotein concentration in *P. purpurea* blade tissue showed approaching saturation (Fig. 3). Half saturation values at occurred at ca. 120 μM NH_4^+. Unlike phycobiliprotein content, tissue N content did not appear to be close to an asymptote, even when grown at 300 μM (Fig. 4).

Preliminary results for *P. purpurea* demonstrate its efficacy as a bioremediator of eutrophic effluent (Fig. 5). During 3.5 d batch cultures, *P. purpurea* removed on average 96-98 % of the NH_4 in the incubation medium. This

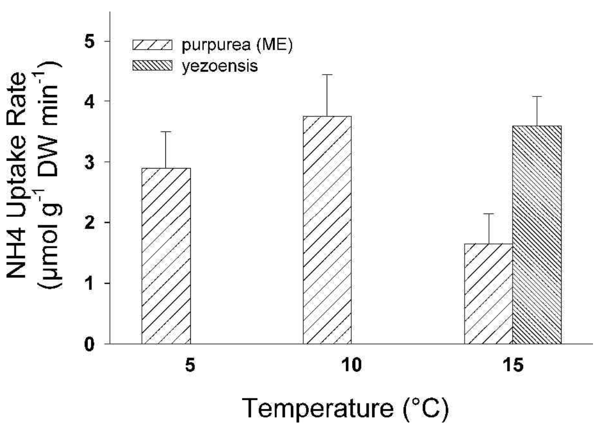

Fig. 1. Short-term NH_4^+ uptake rates measured as a function of temperature. All measurements conducted at 150 μM ammonium. Errors bars are standard deviations.

removal declined to 77 % at 300 μ M. Interestingly, the efficiency with which *P. purpurea* removed inorganic P declined as N concentrations increased (molar N:P ratios constant at 10: 1). At 150 μ M NH_4 and 15 μ M PO_4^{3-} *P. purpurea* removed 46 % of the inorganic P.

Discussion

The short-term measurements of NH_4 uptake rate demonstrated interspecific differences in performance. Clearly, not all *Porphyra* species are equally efficient in the uptake of NH_4, an argument for the evaluation of a range of possible species before selecting the best bioremediator. *Porphyra yezoensis* outperformed *P. purpurea*, at least at 15 ℃, but we do not yet know whether other local species will compare with the Asian species. Some have argued that short-term uptake measurements are to some extent artifactual due to "surge" uptake (e.g., Dy and Yap, 2001). However, previous work with *P. yezoensis* suggests that this phenomenon is not common to all macroalgal species (Yamamoto, 1992). The fact that a definite temperature optimum was identified for one *Porphyra* species suggests that further ex-

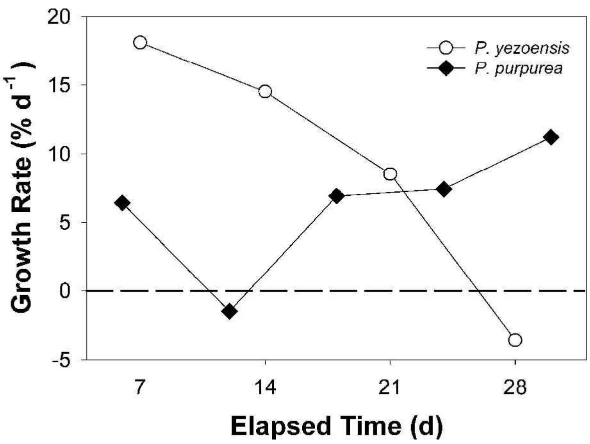

Fig. 2. Average growth rates (n=3) during long-term experiments (150 μ M NH_4). Negative growth rates occur when tissue becomes reproductive and fragments

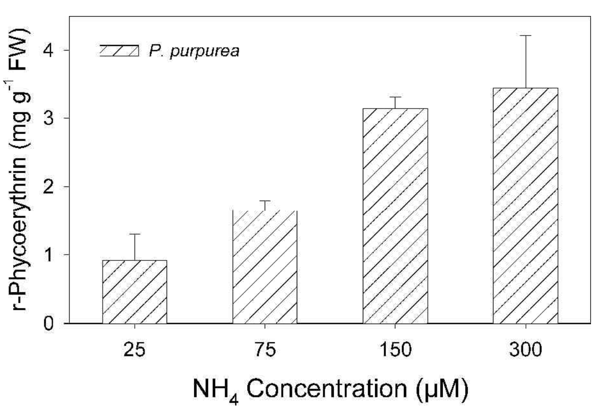

Fig. 3. Ammonium concentration vs. *Porphyra purpurea* r-phycoerythrin concentration at end of 28-d experiment (n=3; error bars are standard deviations)

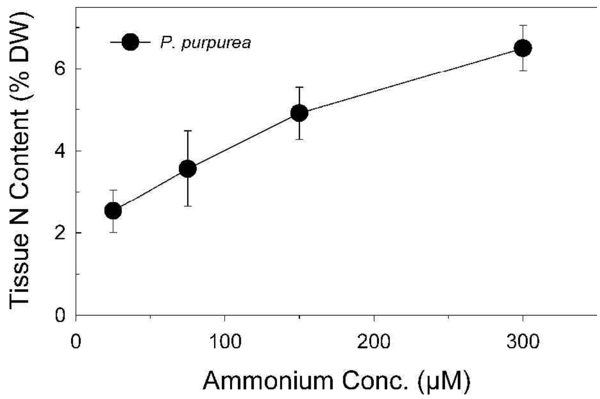

Fig. 4. Relationship between N availability (as NH_4 concentration at start of each 3-4 d growth period) and tissue N content at the end of the 28-d experiment (n=3; error bars are standard deviations)

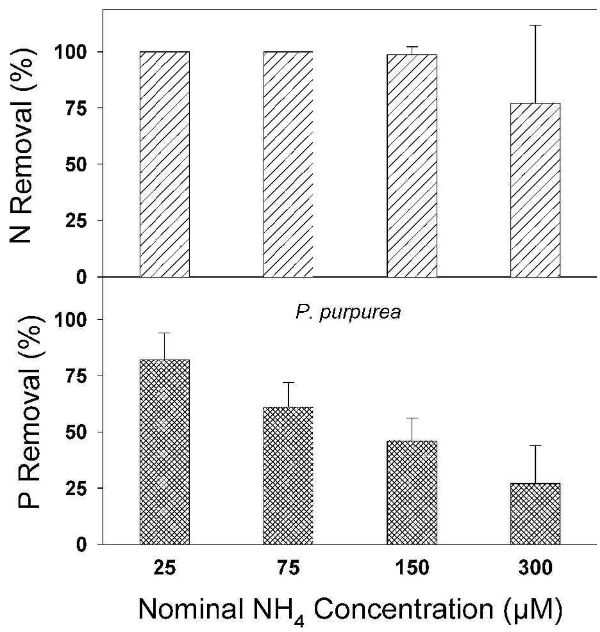

Fig. 5. Efficiency of nutrient removal from incubation media. Values are averages over entire 28-d experiment (n=12; error bars are standard deviations)

panding the pool might provide a high- or low temperature tolerant species. This would enable seasonal crop rotation to take advantage of temperature optima for each particular species. Alternatively, project engineering could be employed to moderate the differences between the operating and optimal temperatures for bioremediation of nutrient-rich aquaculture effluent.

When grown during the long-term experiments (effectively 3.5 d batch culture at 15 ℃, 150 μmol photons m^{-2} s^{-1}, and a stocking density of 0.4 g FW L^{-1},) the performance indicators indicated that different physiological functions respond differently to the availability of inorganic nitrogen. Data for *P. purpurea* regarding phycobiliprotein concentration indicates that production of pigments is not restricted under our experimental conditions in the same way that growth is limited. That pigments continue to accumulate under nutrient-replete conditions suggests that these compounds can act both as light absorbers and as nitrogen stores, something reported for other red macroalgae under N-sufficient conditions (Bird *et al.* 1982; Vergara *et al.*, 1995). Presumably, as growth causes an increase in biomass density the extra pigments are advantageous in light of the diminution of the irradiance regime. Second, tissue nitrogen accumulates in an apparently linear function of nitrogen availability, with no indication of saturation even at 300 μM NH_4. This argues that nitrogenous compounds other than pigments are important in the storage of nitrogen. Amino acids are a candidate. Our unpublished data demonstrate such a response to nitrogen availability, though the increases in amino acids concentrations may not account for all of the increase in tissue nitrogen.

Our short-term data indicates that *P. purpurea* (Maine isolate) is capable of removing ca. 120 μmols NH_4 g^{-1} FW d^{-1} (assuming 10: 1 FW: DW ratio and uptake during 12 lighted hours of a day). The long-term experiment using *P. purpurea* (New York isolate) indicates an average uptake rate during a 3.5 d period of 66 μmols NH_4 g^{-1} FW d^{-1}. This difference may be due to true differences in performance between strains. However, the average uptake rates during the long-term experiments reflect an integration of the effects of changing NH_4 concentration during the 3.5 d period. The uptake rates in a functioning, flow-through system will be much higher, since the concentration will be correspondingly higher. The actual bioremediation (i.e., % N and P removed) will depend on *Porphyra* stocking density and effluent residence time, in addition to concentration-specific uptake rates. These are variables to be investigated at larger scales.

Acknowledgements

Support for this project was provided by funding to C. Y., C. N., and G. P. K. by the Sea Grant Programs of Connecticut, New Hampshire, and New York, respectively and by NOAA's National Marine Aquaculture Initiative, U.S.A.; to C. Y from the State of Connecticut Critical Technology Program; and to X. T. by the National Natural Science Foundation of China for Science.

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水研センター研報，別冊第１号，83－90，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 83-90, 2004

An assessment of the beneficial roles of *Nannochloropsis oculata* in larval rearing of marine finfish

Masanori OKAUCHI*

Abstract From points of both water quality management by algal nutrient absorption and a good live food of rotifer, the role of *Nannochloropsis oculata* supplied to larval rearing water was examined by a larval rearing experiment in this study. Larvae of Japanese red sea bream, *Pagrus major*, were reared being fed rotifers and subsequently an artificial diet during the thirty-day experimental period. In the four test tanks (100-L capacity) each with 1,000 Japanese red sea bream larvae in addition to the rotifers, *N. oculata* was maintained at a density of 5-10 $\times 10^5$ cells/mL while in the four control tanks, no *N. oculata* supplemented, but all the other experimental conditions were the same as the test tanks.

At the end of the experiment, the concentrations of inorganic nitrogen (NH_4-N, NO_2-N, and NO_3-N) and inorganic phosphate (PO_4-P) in the control tanks were 1.5-2.0 times higher than those in the test tanks. While, the number of rotifers with eggs in the test tanks was remarkably higher in comparison with that in the control tanks. Moreover, rotifers in the test tanks contained much more eicosapentaenoic acid (EPA) and n-3 highly unsaturated fatty acid (n-3 HUFA), so that their nutrient value is concluded as higher than that in the control tanks. From the results of this experiment, supplementation with *N. oculata* into the rearing water is concluded to produce beneficial effects on maintaining water quality and also enhancing the nutrient quality of the rotifers.

Key words: *Nannochloropsis oculata*, larval rearing, water quality management, *Pagrus major*, rotifer, *Brachionus*, food organism

Recently, the condensed freshwater *Chlorella* has been popularly used as a food organism for L-type and S-type rotifers, *Brachionus plicatilis* and *B. rotundiformis*, in Japanese hatcheries of marine fishes (Hirayama *et al.*, 1989; Maruyama *et al.*, 1989). Because condensed freshwater *Chlorella* is readily available from several private companies, it has contributed to much laborsaving and allowed the realization of the stable production of rotifers (Yoshimura *et al.*, 1997). While, the number of hatcheries which culture the microalga *Nannochloropsis oculata*, which has been used as the main food for rotifers for more than 20 years, has gradually reduced in Japan. However, the freshwater *Chlorella* has mainly three demerits as a live food. (1) *Chlorella* contains little n-3 highly unsaturated fatty acids (n-3 HUFA) (Maruyama *et al.*, 1986), which are essential fatty acids for marine fishes (Watanabe *et al.*, 1983), so its nutritive value has been considered as lower than that of *N. oculata*. (2) The alga does not survive long-term in sea water and readily decays within several days. The dead cells then induce water pollution. (3) The alga has a hard cell wall, so that it cannot be used as a food organism for almost all important aquacultured invertebrates.

2003年６月12日受理 (Received on June 12, 2003)
* National Research Institute of Aquaculture, Fisheries Research Agency, Nansei, Mie 516-0193, Japan

From these reasons, small quantities of *N. oculata* are cultured in many hatcheries and used to enrich the HUFA content of rotifers, which are predominantly cultured with *Chlorella*, before feeding the rotifers to finfish larvae. Moreover, it is recognized empirically that the addition of *N. oculata* into the rearing water is useful to increase the activity and survival of larval fish (Eda *et al*., 1990; Murashige *et al*., 1991; Tamaru *et al*., 1994). In this study, the effect of *N. oculata* additions to the rearing water was investigated from the point of view of both the quality of the food organisms rotifers and for water quality management in the larval rearing tanks.

Materials and Methods

Materials

Japanese red sea bream, *Pagrus major*, was selected as the material of this study, because the larval rearing method is fully established and larval production is carried out regularly in Japan. Eggs were obtained from a matured female, and were fertilized artificially. Prior to the experiment, the larvae were reared for 10 days after hatching in a 500-L polycarbonate tank with a continuous air-supply and water-exchange system. The larvae were fed with sufficient S-type rotifer during this period.

Nannochloropsis oculata collected from Gokasho Bay in 1986, and cultured in liquid Guillard F medium (Guillard and Ryther, 1962) was used in this experiment. This strain has been cultured using 100-mL flasks with periodic changes of medium under 20℃ and 50 μ mol/m^2/s for more than ten years. During the experiment, the alga was produced using some 10-L glass carboys in batch style under conditions of 25 ℃ and 80 μmol/m^2/s. When the algal growth reached the stationary phase, it was added to the larval rearing water.

S-type and L-type rotifers were obtained from Mie Prefecture Fish Farming Center, Hamajima City. They could make few resting eggs in mass production. They mainly reproduced by parthenogenesis and produced few resting eggs in mass production. They were produced using some 500-L polycarbonate tanks fed with freshwater *Chlorella* at 25 ℃. The artificial diet for red sea bream larvae used in this study was purchased from a Japanese private company.

Larval rearing experiment

The experiment was conducted using eight 100-L transparent polycarbonate tanks each with 90-L filtered seawater. Four tanks were used as test trials and others were used as control. One thousand larvae at age 10 days were introduced into each tank. In the test tanks, both *N. oculata* and rotifers were put into the rearing water at densities of 10^6 cells/mL and 10N/mL, respectively, at the beginning of the experiment. They were kept at 5-10 $\times$ 10^5 cells/mL and 10-20/mL, respectively, during the experiment. In the control tanks, the density of rotifers was kept almost the same as that in the test tanks, however, *N.oculata* was not supplied. S-type and L-type rotifers were fed from day 10 to day 20 and from day 20 to day 30, respectively. Before being supplied to the larvae, the rotifers were fed with *N. oculata* sufficiently for one day to enhance the eicosapentaenoic acid (EPA) levels. From day 30 to the end of experiment (day 40), the appropriate quantity of artificial diet was fed in each tank.

The densities of *N. oculata* and rotifers in all tanks were estimated twice daily using a coulter counter and a stereoscopic microscope, respectively. Moreover, the densities were adjusted to the fixed densities by further additions or by draining and adding filtered seawater. The rearing water temperature was kept at 20-22 ℃ and aeration was continuously supplied at a rate of 400-500 mL/min per a tank. To maintain the water quality in each tank, about 30-L rearing water was exchanged once a day before feeding using thin vinyl tubes.

Counting of dead larvae and female rotifers carrying eggs

Dead larvae, which sank to the bottom of each tank, were collected each day into a white beaker using thin glass tubes with a siphon effect. The average and standard deviation (±S.D.) were calculated from the number of dead larvae in the four test tanks and four control tanks, respectively. Moreover, four 10-mL samples were taken from each tank in order to cal culate the amictic (undergoing parthenogenesis) female ratio (%); (the number of rotifers carrying eggs/the number of rotifers) × 100. Because no resting eggs were observed in the mass culture tanks of S-type and L-type rotifers, all rotifers carrying eggs were regarded as amictic females in this experiment.

Water quality analysis

In this experiment, the contents of inorganic nitrogen (total ammonia; NH_4-N, nitrite nitrogen; NO_2-N, nitrate nitrogen; NO_3-N) and inorganic phosphate (PO_4 -P) were selected as indicators of water quality. Water samples (about 500-mL) for their analysis were collected from each tank every day before changing the water. The samples were filtered using GF/C (Whatman). The filtered samples were put in 500-mL polyethylene bottle and preserved at −30 ℃ for later analysis. The total ammonia was measured using an ammonia electrode with digital ion analyzer (Orion Research, model 701A). Other nutrients were measured according to the methods of Strickland and Parsons (1972).

Measurement of the survival rate of larvae

At the end of the 30-day rearing experiment, the number of surviving larvae and the total body length of 100 randomly sampled larvae from each tank, were measured and their aver ages±S.D. in the test tanks and control tanks were calculated and analyzed by one-way ANOVA.

Fatty acid analysis of rotifers

On day 30, at the start of feeding the artificial diet, rotifers in the test and control tanks were sampled using a plankton net. They were washed with fresh water for about ten minutes, and were stored at −80 ℃ until analysis. Furthermore, rotifers enriched with *N. oculata* after being produced with freshwater *Chlorella* were also analyzed. Total lipids were extracted by chloroform-methanol method according to Folch *et al.*, (1957). The fatty acids of the total lipids were methyl-esterified and analyzed by gas-liquid chromatography.

Results

Survival and growth of larvae

Daily changes of the average number of dead larvae in the test and control tanks are shown in Fig. 1. The number of dead larvae per day in the control tanks was significantly higher than that in the test tanks after day 28 ($P<0.01$). The average total number of the conformed dead larvae in test tanks during this experiment was about 100, while that in the control tanks was about 198. The averages±S.D. of the total body length (mm) in the test and control tanks were 9.5±0.9 and 8.8±1.2, respectively. Thus, larvae in the test tanks were significantly larger ($P<0.05$) than those in the control tanks.

Rate of rotifers with eggs

Daily changes of the percentage of rotifers with eggs in the test and control tanks are shown in Fig. 2. Regardless of the rotifer type, the number of rotifers with eggs in the test tanks was clearly higher than that of the control tanks. More than 20 % of rotifers in the test tanks carried one or two eggs under their loricas, while rotifers with eggs in the control tanks were less than 10 %. During the experiment, no mictic reproduction was observed in any tank.

Fatty acid components of the rotifers

Percentage composition of the fatty acids of rotifers is shown in Table 1. The crude protein and crude lipid contents of them are also shown. The rotifers collected from the test tanks contained much EPA comparison with the rotifer from the control tanks.

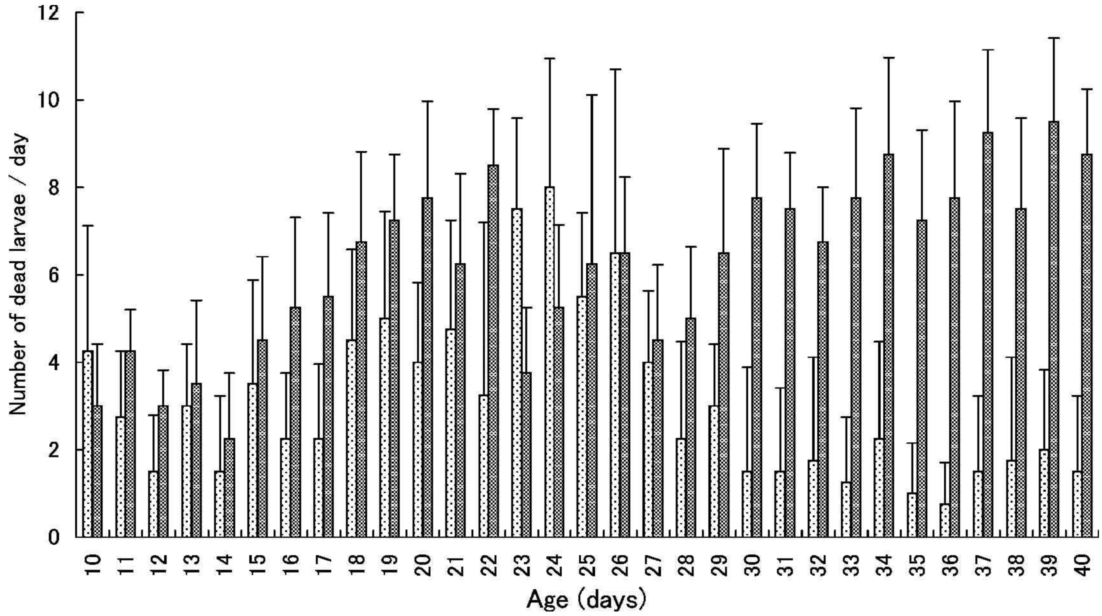

Fig. 1. Daily changes of the average number of dead larval Japanese red sea bream, *Pagrus major*, in the test tanks (Dotted bars ; ⬚) and the control tanks (Shaded bars ; ▩). Vertical lines indicate the standard deviations. X-axis shows the larval age, which is indicated by days after hatching.

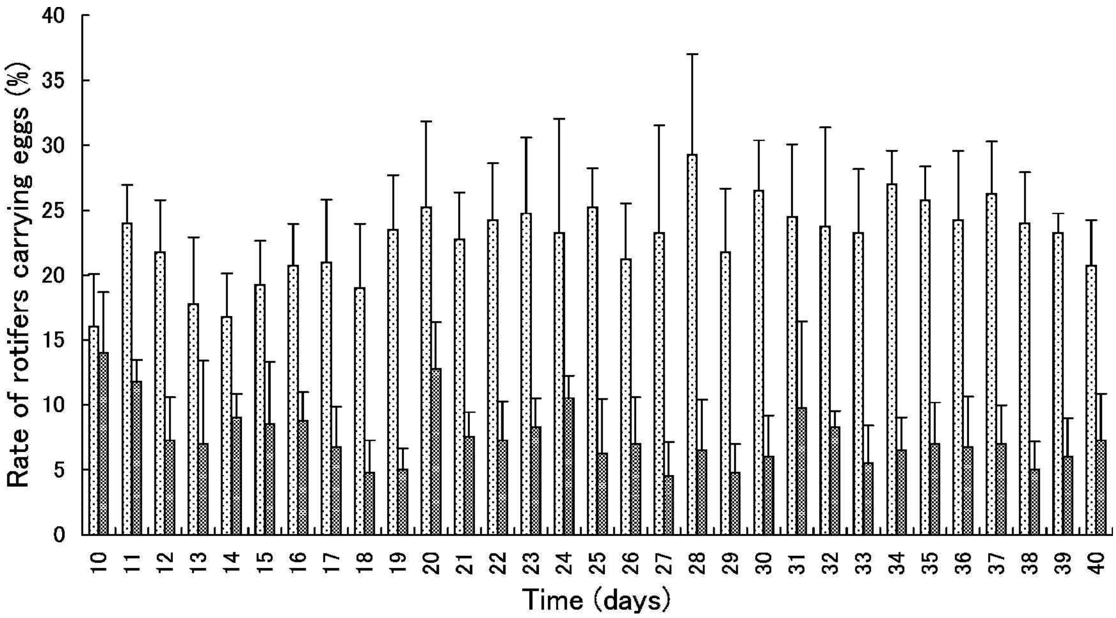

Fig. 2. Daily changes of percentage of rotifers with eggs in the test tanks (Dotted bars ; ⬚) and the control tanks (Shaded bars ; ▩). Vertical lines indicate the standard deviations. X-axis shows the elapsed time from the larval hatching out. In order to calculate the ratio of females carrying eggs, four 10-mL samples were taken from each tank and the number of rotifers with eggs was counted using a stereoscopic microscope.

Consequently, the content of n-3 HUFA contained in rotifers from the test tanks was significantly higher than that in rotifers from the control tanks. While, the fatty acid component of rotifers, which were secondarily enriched with *N. oculata* after production using freshwater *Chlorella*, was almost the same as that of rotifer from the test tanks. On the other hand, the protein content of rotifers from the control tanks was lower in comparison with those of rotifers from the test tanks.

Table 1. Percentage composition of the fatty acid components, percent total lipid and crude protein of rotifers collected from the test tanks and the control tanks are shown in the first and second rows. Moreover, these components of rotifers, which were secondarily enriched with *N. oculata* after production using freshwater *Chlorella*, are shown in the third row for comparison.

(%)

Fatty acid	Rotifers in the test tanks (with *Nannochloropsis*)	Rotifers in the control tanks (without *Nannochloropsis*)	Rotifer cultured with *Chlorella* (secondary enriched with *Nannochloropsis*)
14 : 0	5.5	5.2	5.8
16 : 0	15.2	15.6	16.4
16 : 1	18.8	11.1	19.8
16 : 4 n-3	0.6	1.2	0.8
18 : 0	2.6	2.2	2.8
18 : 1	8.2	9.6	10.5
18 : 2 n-6	4.5	5.6	4.7
18 : 3 n-6	0.5	0.7	0.4
18 : 3 n-3	0.5	0.2	0.8
18 : 4 n-3	0.9	0.8	0.1
20 : 1	3.7	2.7	4.7
20 : 2 n-9	0.2	0.2	0.3
20 : 3 n-6	0.4	1.1	1.0
20 : 4 n-6	3.2	2.7	4.7
20 : 4 n-3	0.5	0.6	0.5
20 : 5 n-3	18.9	5.8	20.8
22 : 1	1.2	2.0	1.1
22 : 3 n-6	0.1	0.3	0.1
22 : 5 n-6	1.6	Tr*2	0.1
22 : 5 n-3	2.6	1.2	5.6
22 : 6 n-3	0.4	0.2	0.4
24 : 1	0.3	0.2	0.4
Σn-6	10.3	10.4	11.0
Σn-3	24.0	10.0	29.0
Σn-3HUFA	28.7	12.5	33.0
Lipid (%) *1	2.5	2.1	2.9
Protein (%) *1	7.2	5.6	7.8

*1 Wet matter basis; *2 Tr indicates trale level

Results of water quality analysis

Daily changes of NH_4-N, NO_2-N and NO_3-N concentrations are shown in Fig. 3. After day 21, the concentration of NH_4-N in the test tanks was significantly lower than that in the control tanks ($P<0.01$), except on days 31 and 32. The average concentration of NH_4-N after day 32 in the test tanks was 1.6 mg/L, while that in the control tanks was 2.2 mg/L. In the case of NO_2-N, its concentration in the test tanks was significantly lower than that in the control tanks after day 30 ($P<0.01$), except on day 33. The average rate of increase per day from day 29 to day 40 in the test tanks was 0.064 mg/L, while that in the control tanks was 0.095 mg/L. The concentration of NO_3-N was about 10 times higher than that of NH_4-N and NO_2-N during the experiment in both test and control tanks. The difference between the concentration of NO_3-N in test tanks and that in the control tanks was significantly different after day 18 ($P<0.01$), except day 21. The average rate of increase per day after day 18 to day 40 was 0.545 mg/L in the test tanks and was 1.002 mg/L in the control tanks.

On the other hand, daily changes of PO_4-P concentration are shown in Fig. 3. No difference between its concentrations in the test tanks and those in the control tanks was detected from day 10 to day 29. However, the difference between them gradually became clear after day 30 ($P<0.01$). At the end of the experiment, the concentration of PO_4-P in the control tanks was about two times higher than that in the test tanks.

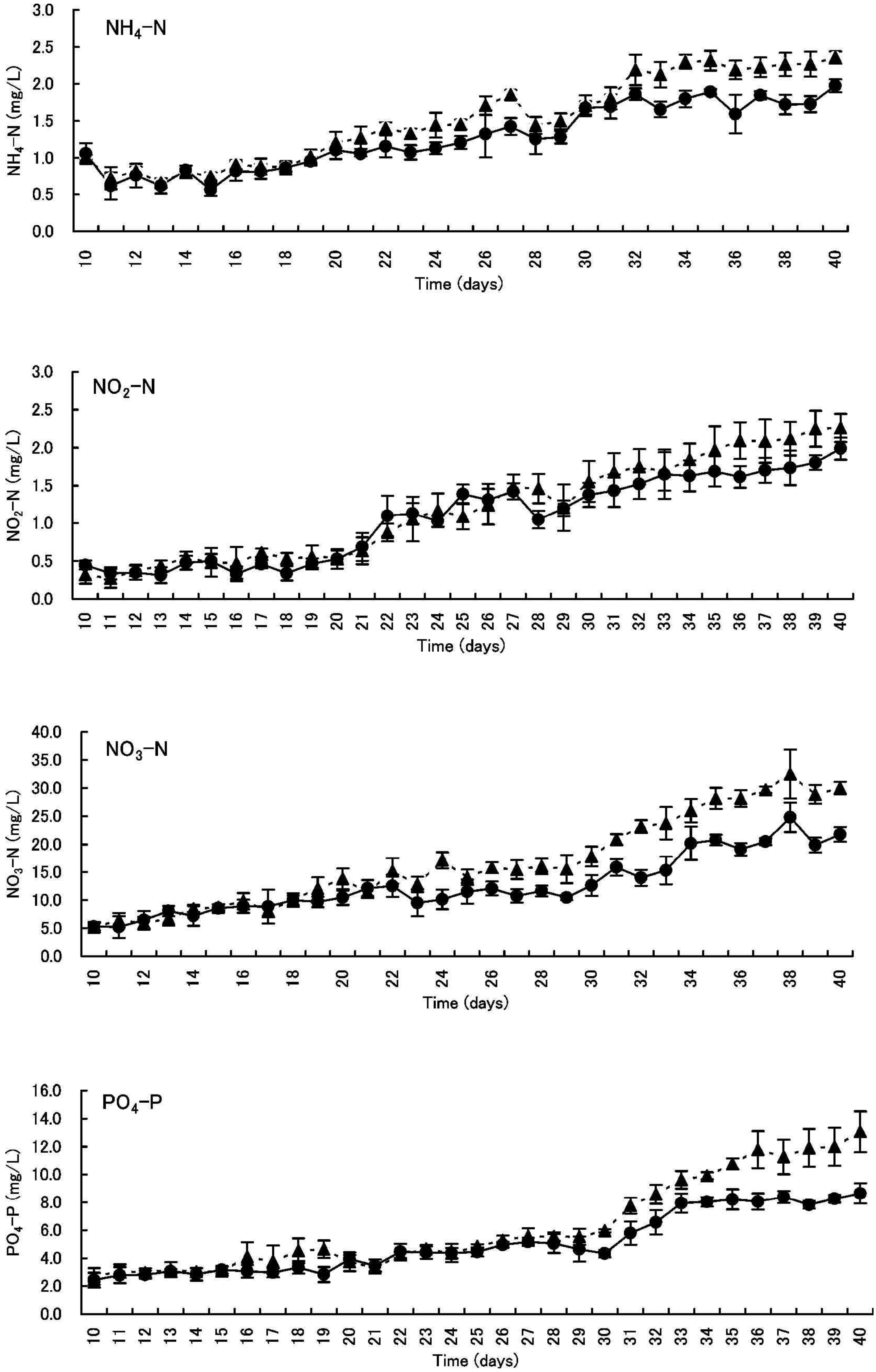

Fig. 3. Daily changes of the average contents of NH_4-N, NO_2-N, NO_3-N and PO_4-P in the test tanks (●) and the control tanks(▲). Vertical lines indicate the standard deviations. S-type rotifers were fed from day 10 to day 20, L-type rotifers were fed from day 20 to day 30, and an artificial diet were supplied from day 30 to the end of the experiment.

Discussion

Efficiency of *N. oculata* to enrich rotifers in larval rearing tanks

Regardless of the rotifer type, the nutritive value of rotifers collected from the test tanks was high comparison with that of rotifers from the control tanks. Especially, the EPA and n-3 HUFA contents of rotifers were reduced clearly in the control tanks. This is concluded to be caused by the deficiency of *N. oculata* in the control tanks. Both types of rotifer put in the control tanks and their newly hatched off-spring were not supplied with alga and as such had minimal available food materials so that their n-3 HUFA and protein contents were reduced compared the test tank rotifers. Even if the larvae are fed such rotifers enough, their survival and growth rates will undoubtedly be lower. While, the rotifers in the test tank fed *N. oculata* enough, so that it's nutritive value was enhanced. Thus, the larvae in the test tanks grew well and their survival rate was high.

Moreover, the rotifers in the test tanks had a higher rate of egg carrying than that in the control tanks. The amictic reproduction of rotifers would be induced more frequently in the test tanks, because of the higher food availability in the tanks. Therefore, the addition of *N. oculata* seemed to be effective to produce both nutritive and reproductively active rotifers in the larval rearing water.

Water quality management by *N. oculata*

After day 30, the number of dead larvae in the control tanks became conspicuously higher than that in the test tanks. However, the same quantity of artificial diet was supplied to both the test and control tanks from day 30. Therefore, there was no difference in the food supplied to the test and control tanks. As the cause of larval death in the control tanks, two possible causes are apparent.

One is the sudden diminution of rotifers in the rearing water. No rotifers were supplied into both the test and control tanks after day 30. However, the number of rotifers only reduced gradually in the test tanks, because active reproduction occurred continuously. Thus, the larvae in the test tanks could eat not only the artificial diets, but also the rotifers. While, the larvae in the control tanks after consuming the remaining rotifer ate the artificial diet only. Therefore, the effect of the addition of *N. oculata* into the rearing water seemed to continue after even changing to the artificial diet.

Moreover, water pollution was a probable factor inducing larval mortality. After changing from rotifers to the artificial diet, inorganic nitrogen (NH_4-N, NO_2-N, and NO_3-N) and phosphate (PO_4-P) concentrations increased both in the test and control tanks. Especially, they increased rapidly in the control tanks. One of the reasons for the differences in concentrations of nitrogen and phosphate in the rearing water between in the test tanks and in the control tanks seemed to algal nutrient absorption. *Nannochloropsis oculata* absorbed dissoluble nitrogen and phosphate as nutrients in the test tanks. Additionally, many larvae died in control tanks, and their bodies, prior to being removed each day would also deteriorate the quality of the rearing water. Thus, water pollution seemed to be accelerating in a shot term. From this point of view, additive *N. oculata* was effective.

In an almost similar rearing experiment using mullet larvae, Tamaru *et al.*, (1994) reported that the NH_4-N content in the test tanks was significantly higher than the control tanks. They considered that the reason was contamination from the algal medium, because they used ammonium sulfate as a nutrient in the medium. In this experiment, sodium nitrate was used instead of ammonium sulfate, so that the toxicity of unionized ammonia was concluded as negligible. Moreover, *N. oculata* was put into the rearing water after the algal growth reached the stationary phase. At the stationary phase, almost all the nutrients in the medium were used for the algal growth. Thus, contamination from the algal medium would not raise the nitrate nitrogen level in the larval rearing water.

A new role of *N. oculata* for larval rearing

From the results of this experiment, it was shown that *N. oculata* has main two roles in the larval rearing water. The addition of *N. oculata* into the rearing water was indispensable to enhance the fatty acid component of rotifers to a level suitable for the fish larvae. Moreover, the addition of the alga was effective to reduce the concentrations of the soluble nitrogen and phosphate. Especially, after feeding with the artificial diet, the concentrations of NO_3-N and PO_4-P increased remarkably. However, because the larvae still only had a weak swimming ability, we could not adopt a continuous flowing system with a high flow rate. When a semi-continuous changing water system was adopted, the alga seemed to be useful as absorbing potentially harmful excess nutrients and contribute to maintain the water quality management.

In the near future, various kinds of artificial diets will be used commonly in larval rearing. Moreover, freshwater *Chlorella* is now usually used in rotifer production. The characteristics of *N. oculata* have significant potential to enhance and stabilize production in finfish larval culture and as such for the effective utilization of this useful link in the food chain in hatchery production, further detailed research is required.

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水研センター研報，別冊第１号，91－100，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 91-100, 2004

Integrating Intensive Aquaculture of the Red Seaweed *Chondracanthus exasperatus*

J. Robert WAALAND*

Abstract Washington State has a significant history of experimental seaweed aquaculture. Early experiments involved the carrageenophytes *Mazzaella splendens* and *Chondracanthus exasperatus* in both open water net culture and semi-closed tank systems on land. Later, net culture of *Porphyra* for nori and long line culture of the edible kelps *Nereocystis* and *Macrocystis* were tested successfully. Further development of these culture systems was halted due to combinations of market, regulatory, political and social considerations. When a Seattle company developed a high value product from the Turkish Towel Seaweed, *Chondracanthus exasperatus*, there was renewed interest in intensive tank and pond based aquaculture because Washington has a long term moratorium on commercial seaweed harvesting from wild seaweed populations. The initial phase of this research was conducted at Mukilteo, Washington, where strategies for long term cultivation in tanks were tested, and a new custom cultivation tank design was developed for pilot scale cultivation research at a larger site on the shore of Clam Bay near Manchester, Washington. Long term cultivation is now being tested in tanks of up to 5,000L volume supplied with natural seawater, nutrient supplemented seawater, and seawater effluent from nearby fish culture tanks. Seawater from Clam Bay is naturally rich in nutrients from tidal driven upwelling and nearby commercial salmon aquaculture operations. Supplemental nutrients (commercially available "f/2" enrichment and agricultural fertilizers) and halibut culture tank effluent have both been tested for their ability to support *C. exasperatus* growth with relatively low seawater turnover rates. Compared to seawater at the Clam Bay site, halibut tank effluent differs in both nutrient composition and quantities. Initial results indicate that halibut tank effluent is a satisfactory source of nutrients for *C. exasperatus* in intensive culture and that this seaweed scrubs significant quantities of nutrients from halibut tank effluent, especially ammonium. Recent experiments with several bioreactor designs have investigated the culturing of *C. exasperatus* at very high loading densities in recirculated natural and artificial seawater in both submerged and spray culture.

Key words: seaweed, red algae, *Chondracanthus,* aquaculture

Introduction

Washington State has a history of experimental seaweed aquaculture. In the 1970s net and tank cultures of the carrageenan producing red seaweeds *Mazzaella* (then known as *Iridaea*) and *Chondracanthus* (then known as *Gigartina*) were tested. In the 1980s, the emphasis shifted to *Porphyra* species, both Japanese and Washington species which were cultivated on raft nets to produce edible nori (Mumford *et al*., 1985; Merrill & Olson, 1988).

2003年６月12日受理（Received on June 12, 2003）
* Department of Biology, University of Washington, Seattle, Washington 98195, USA Email: jrw@u.washington.edu

Late in the 1980s, long line cultivation of the kelps *Nereocystis* (as a wakame substitute) and *Macrocystis* (for the herring roe on kelp fishery) were tested on long lines (Merrill, 1989). Recently interest in cultivation of *Chondracanthus exasperatus* was renewed when a Seattle company discovered and developed a high value cosmetic product from this seaweed. Since Washington State has a moratorium on commercial seaweed harvesting from natural populations and harvest limits elsewhere in this species' range limit harvests from the wild, aquaculture was viewed as the best option for obtaining raw material to support this developing industry. As the earlier open water aquaculture activities had elicited considerable opposition from shoreline property owners, open water cultivation was not considered a viable option. Therefore, intensive tank or pond cultivation was viewed as the best option for obtaining a sustained raw material supply to support this new industry. Furthermore, intensive aquaculture offers opportunities for optimizing product yield through strain selection, genetic manipulation, control of certain growth parameters and protection from pests and competition. A joint university, industry and agency project was initiated to develop this option.

In earlier research, both open water net cultivation and tank culture of *Chondracanthus exasperatus* had been tested on a limited basis as *Mazzaella splendens* (as *Iridaea cordata*) was the species which received the most attention in the 1970s research (Waaland, 1973, 1976). What was known was that *C. exasperatus* exhibited dependable growth in tank culture and could be cloned and propagated perennially from vegetative fragments. Additional data were available about loading densities, seawater turnover and mixing required for sustainable growth in intensive tank culture (Waaland 1977).

This report includes both field and laboratory aspects of recent research which has progressed through three phases. The first phase investigated strategies for long term maintenance of seaweed stocks and explored experimental and pilot scale tank designs for cultivation of *C. exasperatus*. The second phase focused on increasing stock quantity and quality by selection and management of growth parameters and integrating water from fish (Pacific Halibut) culture tanks. The third and most recent phase has emphasized reducing water use and pumping through recycling and spray culture.

Methods

The *Chondracanthus exasperatus* (Turner & Bailey) J. Hughey plants used to initiate these experiments were obtained from natural populations at Clam Bay near Manchester, Washington in Sept. 1999. After the initial collection, most material used in large-scale experiments came from the tank cultures with occasional use of freshly collected wild plants.

In certain smaller scale laboratory and field experiments, plants from uni-algal laboratory experiments were sometimes used. These were typically maintained in natural seawater enriched with commercially available Guillard's f/2 enrichment (Kent Co. marketed by Aquatic-Ecosystems), grown in controlled environment chambers at 15℃ with irradiance of $\sim 50\,\mu E \cdot m^{-2} \cdot s^{-1}$ from cool white fluorescent lamps (Merrill & Waaland 1979). In many experiments, artificial seawater (Instant Ocean®, Aquarium Systems Inc., Mentor, OH) made with reverse osmosis water to salinity 30o/°° (typical of Puget Sound) and supplemented with Guillard's f/2 enrichment (Kent Co. chemicals as above) was used successfully.

Outdoor Experiments

Several types of tanks were used depending on the experiment. The tanks at the first outdoor site, Mukilteo, Washington were cylindrical fiberglass fish culture tanks 1.2m diameter, 1.0m deep, volume 1500L, with a center drain (Fig. 1). Water motion was provided by a central aerator.

At the Manchester, Washington site, most tanks were horizontal half cylinders with hemispherical ends (4.7m long, 1 m deep, 1.5m wide, 5500L volume) with a well screen equipped drain near each end (only one drain was active, the other was used for instruments, cross connecting tanks and other purposes) (Fig. 2). A longitudinal aeration pipe circulated the seawater and seaweeds. Smaller versions of these tanks were used in a few experiments. An array of ten 75L semi-square polyethylene tanks was used for small scale field experiments; they were equipped with well screen center drains and central aeration (Fig. 3). Seawater was pumped from an intake on a nearby pier (visible in background in Fig. 3). At the Manchester site, effluent seawater from Pacific Halibut culture tanks was also available. Typical salinity is 28-30o/^{oo} at these sites.

Typical tank culture conditions involved ambient daylight and ambient temperatures of the pumped seawater. At this latitude (∼47°N), winter days are short (∼8.5 hr light) and often cloudy and summer days long (16 hr light). Pumped seawater temperatures ranged from 4-6 ℃ in winter to 14-15℃ in summer. Solar heating may raise water temperatures one or two degrees in large tanks depending on flow rate. Light and temperature in or near the large tanks were monitored with data loggers (Onset Computer Corp.). Other experimental details will be given with each type of experiment. Nutrient analyses (nitrate, nitrite, ammonium, phosphate) were performed by the Marine Chemistry Laboratory, Department of Oceanography.

Greenhouse Experiments

In the greenhouse, plants were housed in an hexagonal plexiglass (0.50m long × 0.45m wide, 100L volume aquarium) turned on its side and fitted with a plastic mesh platform to support the plants and equipped with drain openings and a removable panel for sealing the opening and providing for sea water supply and drain pipes (Fig. 4).

Plastic spray nozzles were connected to an external polyethylene reservoir recirculating 80L of UV sterilized (QL-25, Rainbow

Fig. 1. Vertical cylindrical tanks used at Mukilteo field station

Fig. 2. Horizontal half cylinder tanks used at Manchester field station

Fig. 3. Semi-square tanks used for outdoor culture at Manchester and indoor spray culture in controlled environment room

Aquarium Systems), 35 μm filtered (Aquatic Ecosystems VF-125), and chilled sea water. The chiller (Aqualogic 0.23Kw) was equipped with a titanium heat exchanger. The seawater medium was pumped at approximately 12-15L・min^{-1} through the spray nozzles. The chiller maintained the seawater at temperatures between 10-15℃depending on the experiment. From late spring through mid autumn, the greenhouse was covered with 30% shade cloth to reduce the solar heat load. In addition to the greenhouse shade cloth, plastic window screen often was used as a neutral density filter on the tank. The ambient light is supplemented on cloudy days and daylength extended in winter with 600W high pressure sodium greenhouse lamps. Data loggers (Onset Computer Corp.) were used to track temperature and light in this system. This culture unit has a relatively low density of seaweed per seawater volume because of the volume needed to cover the chiller probe in the reservoir. The large volume of chilled medium also adds a brief period of buffer time to react to failure of the cooling system in the high ambient heat of the greenhouse environment. The unit has operated satisfactorily for months at a time with weekly shut offs to remove the plants for weighing.

Typical Operating Conditions:
Light: ambient greenhouse, shading in summer, ～150 $\mu E \cdot m^{-2} \cdot s^{-1}$
Temp: 10-12℃
Nutrients: 0.1×f/2 (low biomass/medium volume)
Seawater: replaced ～7-8weeks
Pumping rate: ～12-15L・min^{-1} (for spray)
Growth rate: ～4-5% d^{-1}

Controlled Environment Rooms

In controlled environment rooms, two types of tanks were used for most experiments: 320L rectangular polyethylene tanks (1.0m×0.67m × 0.65m) (Fig. 5) and 0.6m × 0.6m, 75L semisquare tanks (described above) equipped with a plastic screen platform to support the plants and using a recirculating pump (20L in circulation), 25 watt UV sterilizer (25 watt) and 35 μm particle filter and plastic lawn sprinkler spray nozzles attached to a distribution manifold (Fig. 6). In the controlled environment rooms, the temperature of the circulating water was maintained in a range (10-15℃) encountered by *C. exasperatus* in natural communities in the Puget Sound region during spring, summer and early autumn.

The 320L rectangular polyethylene tanks were used primarily as holding tanks for plants to be used in other experiments. Nevertheless, they demonstrated that high densities of plants may be maintained for up to 16 months in batch culture with little seawater

Fig. 4. Hexagonal chamber used for spray culture in greenhouse environment

Fig. 5. Rectangular plastic tanks used in controlled environment room

turnover (once every 3-6 months) provided that mineral nutrients are replenished and salinity controlled (by addition of reverse osmosis water) as required. For faster growth, addition of carbon dioxide (via a pH controller and sparger) resulted in higher growth rates. Particle filtration (35 μm) and UV sterilization (25 watt) were essential to control phytoplankton species in such a system and to reduce the survival of spores of competing seaweeds (e.g., *Ulva* and *Enteromorpha*, etc.) which might be circulating in the system, introduced with the *C. exasperatus* from natural populations. Growth rates up to 5 % d^{-1} day were observed in this system. It would be relatively simple to integrate this type of culture system with a finfish or shell culture system.

Laboratory Benchtop Bioreactor

Cytolift® Bioreactor (Kontes Glass Co.) vessels were used for the most intensely monitored experiments (Fig. 7). These are cylindrical, water-jacketed bioreactors with an internal volume of ~500mL. Water circulation was provided by a multi-channel peristaltic pump (Cole-Parmer/MasterFlex Model 7553-70). Seawater was chilled in a countercurrent heat exchanger chilled by a 0.12Kw Lauda K-2/R chiller circulating chilled tap water. Circulating seawater was UV treated by circulating the water through quartz tubing in a cylindrical aluminum chimney housing a 25 Watt UV lamp. A computer interfaced data logger (pH, dissolved oxygen, temperature and light) and controller (Remote Measurement Systems, Inc., "EnviroMac" unit) was used to monitor experimental conditions and to add CO_2 as the plants consumed it via photosynthesis.

Fig. 6. Semi-square tank with recirculating pump, UV sterilizer and particulate filter as used for spray culture in controlled environment room

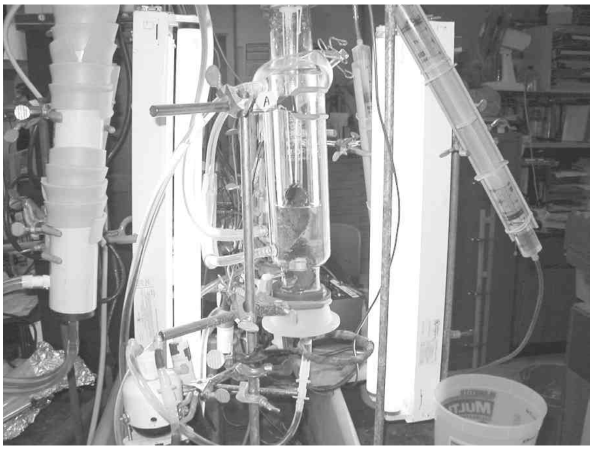

Fig. 7. Cytolift® bioreactor used in laboratory

An Olympus 620L digital camera was used to record the appearance of individual tagged plants at weekly intervals. Fresh weights were measured after plants were drained and blotted.

Results

The results presented here are representative of the performance, as measured by relative growth rate, of plants raised under the conditions specified for each series. These represent the potential performance of such systems but do not detail experiments that were not productive.

Outdoor tanks

At present, the large outdoor tanks

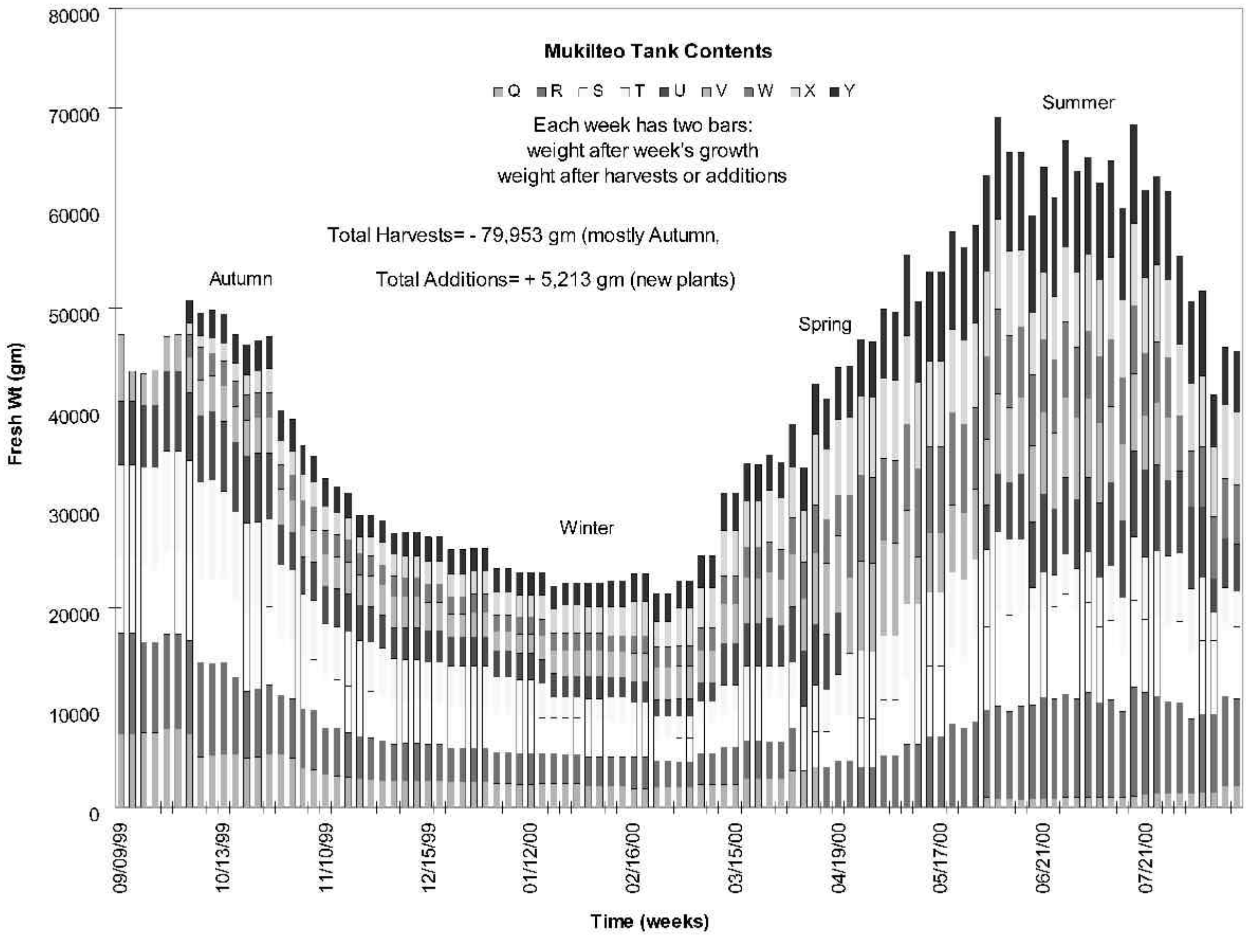

Fig. 8. Summary graph for Mukilteo tank array

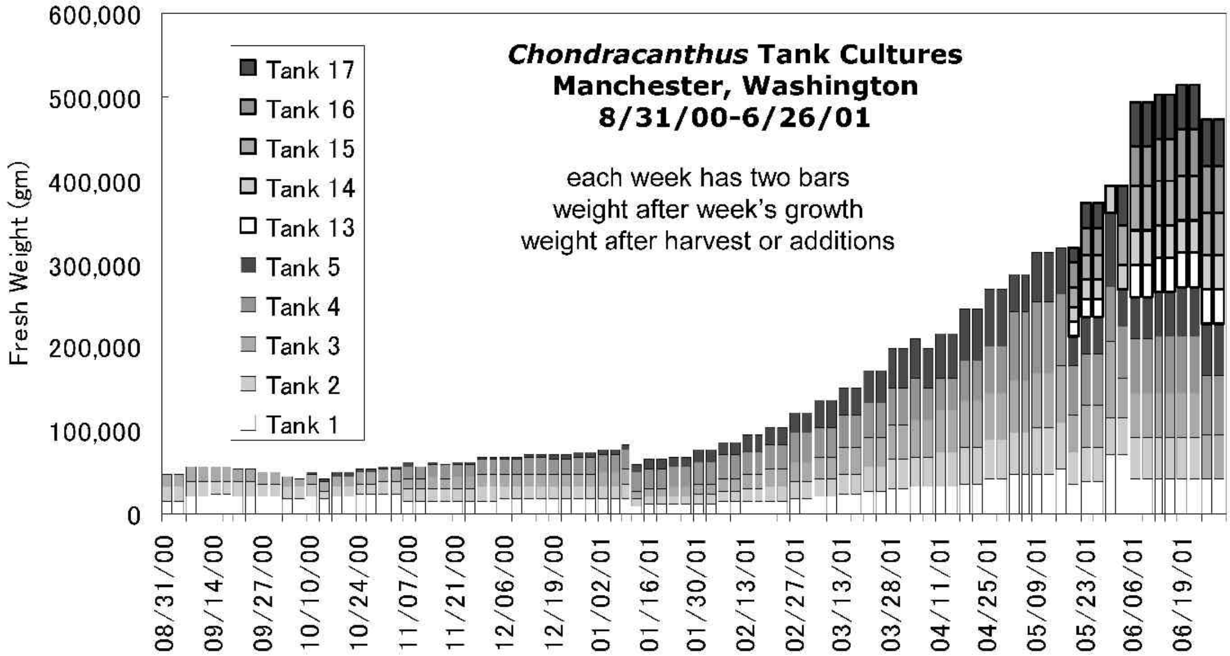

Fig. 9. Summary graph for Manchester tank array

represent the most realistic option for pilot or small commercial scale production. Several years of experience have been obtained encompassing all seasons and a variety of weather conditions (from September 1999 through late August 2000 at Mukilteo and late August 2000 through mid-June 2001 at Manchester for our experiments and subsequently to the present by Söliv personnel at Manchester).

A summary of production in the Mukilteo tanks is seen in Fig. 8. A similar summary for the Manchester large tank array is seen in Fig. 9.

For some experiments some or all of the Manchester tanks were supplied with effluent water from adjacent halibut rearing tanks. Fig. 10 shows the ammonium supplementation that occurred as a result of the halibut in the water and the reduction in ammonium that occurred as a result of *C. exasperatus* growing in the water.

Greenhouse experiments

Several tank and container designs were tested in greenhouse conditions, but only one is shown here, the hexagonal enclosure used for spray culture (Fig. 4). Early experiments did not use particle filtration or UV sterilization of the medium. Such filtration (to prevent nozzle clogging) and sterilization (to inhibit epiphyte and phytoplankton growth) proved essential for long term operation (months) of culture units using recirculated medium. Without such treatment, diatoms, green flagellates and ulvoids were recurring problems. Typical operating conditions for the recirculating hexagonal enclosure system were:

Light: 150 μ E・m^{-2}・s^{-1} (ambient greenhouse light, shaded and/or supplemented with sodium vapor lamps)
Temp: 12℃
Nutrients: 0.15×f/2/week
Seawater: replaced ∼every 8-20 weeks
Pumping rate: 12L・min^{-1} for circulation and spray operation
Growth rate: ∼1-2% d^{-1}

A representative plant growing in this system is shown in Fig. 11.

Controlled Environment Room Experiments

Controlled environment rooms provide a convenient way of maintaining seaweed cultures at temperatures similar to those of their natural environment. UV sterilization of the seawater medium pumped using an airlift system, proved essential to prevent growth of competing algae (e.g., diatoms, green flagellates and ulvoids). With a pH controller to add carbon di-

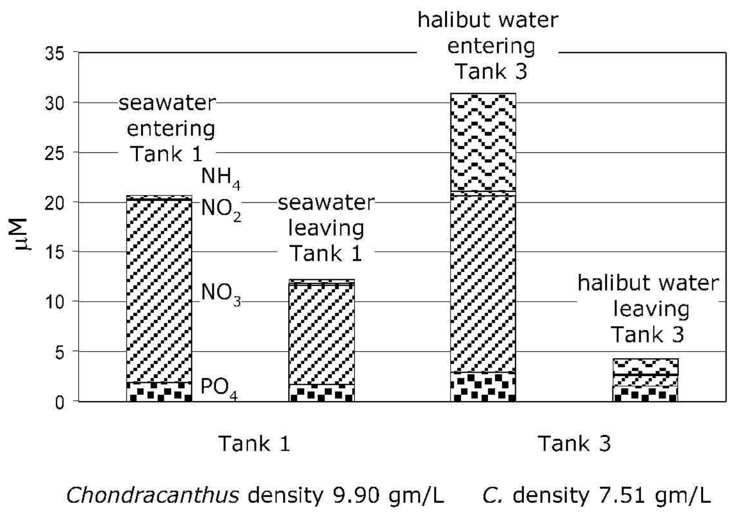

Fig. 10. Seawater and halibut tank inflowing and effluent water. Samples were taken near mid-day. Loading density was as shown. Note the preferential use of ammonium over nitrate. Plants were actively growing although the plants in Tank 1 were at a very high density and may have been light limited compared to those in Tank 3

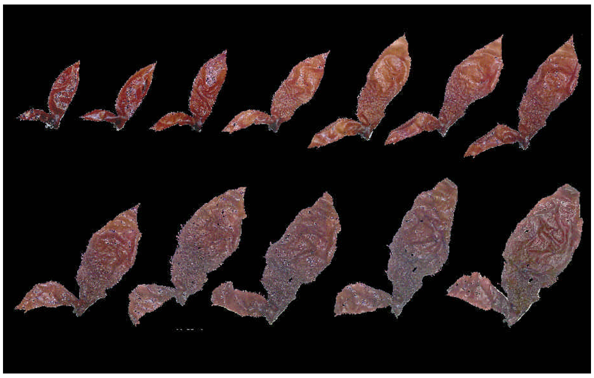

Fig. 11. Representative plant grown in spray culture in hexagonal enclosure in greenhouse

Fig. 12. (Top): Representative plant ER-3 grown in spray culture in controlled environment room (Bottom): Representative plant 02-05-31 growing in benchtop Cytolift Bioreactor

oxide as the pH rose above 8.2 and mineral nutrient supplementation by periodic additions of f/2, it was possible to maintain plants in an actively growing state at very high densities for several months in the same batch of seawater. Typical operating conditions for the 320L tanks follows:

Light: 150 $\mu E \cdot m^{-2} s^{-1}$ (metal halide and cool white fluorescent lamps; 16 hr L: 8 hr D)
Temp: 12℃
Nutrients: 0.15×f/2/week
Seawater: replaced ∼every 8-20 weeks
Pumping rate: aeration for circulation, air lift pump for UV sterilizer
Growth rate: ∼1-2% d^{-1}

Semisquare Spray Culture System

This system has a very small volume of water (∼20L) circulating in a chamber equipped with a plastic mesh rack to support plants and plastic spray nozzles to keep the plants damp and supply mineral nutrients. This system circulates the seawater medium through a UV sterilizer to inhibit competing algae and a particle filter which prevents fouling of the spray nozzles and also removes competing algae. This system has proved quite successful.

Typical Operating Conditions:
Light: 150 $\mu E \cdot m^{-2} s^{-1}$ (metal halide and cool white fluorescent lamps; 16 hr L: 8 hr D)
Temp: 12℃
Nutrients: 1.5×f/2/week
Seawater: replaced ∼every 4-10 weeks
Pumping rate: ∼12L/min for spray
Growth rate: ∼4-5%/d
A representative plant is shown in Fig. 12 (top).

Cytolift Bioreactor

The Cytolift® Bioreactor units have a very high plant density per volume of circulating medium. The temperature and light monitors mainly reported on the functioning of the lamp timer and circulating chiller. The pH monitor could add CO_2 to the closed system via a low volume pumping system. The oxygen monitor was useful only when plants were entirely submerged with no air space to provide an oxygen reservoir and sink. Most useful for these experiments was the pH monitor and regulator as it provided rapid feedback and also compensated for carbon dioxide uptake by rapidly growing plants. Mineral nutrient additions were done manually with the frequency and amount depending on the experiment being conducted. Similar growth rates are obtained for immersed or emersed plants. A representative plant is shown in Fig. 12 (Bottom).

Typical Operating Conditions:
Light: 150 $\mu E \cdot m^{-2} s^{-1}$ 16L: 8D & 20L: 4D (fluorescent)
Temp: 15-20℃
Nutrients: pH maintained at 8.0-8.2; f/2 enrichment 1.2×per week (proportional to biomass)
Seawater: replaced ∼every 4 weeks
Pumping rate: ∼0.2L/min
Growth rate: ∼4-5%/d

Discussion and Conclusions

This report describes several scales of culture method for use with the red seaweed *Chondracanthus exasperatus*. Many other seaweeds are likely to flourish in such systems as well. The larger scale flow through outdoor systems are presently used as a pilot scale research system for commercial production associated with a high value product extracted from *Chondracanthus*. The smaller scale experimental systems can be modified for immersed or emersed culture of this seaweed and probably other seaweeds. All the tested systems offer opportunities for reducing the volume of seawater used. All the laboratory scale systems have been successfully tested with both natural and artificial seawater medium. Recirculation offers significant economies in reduced pumping and materials handling costs. All tested systems benefit from nutrient supplementation, particularly at low seawater turnover rates. Mineral nutrient supplementation is essential in low volume and/or turnover systems to maintain plant growth. Carbon dioxide supplementation improves growth rate where CO_2 limits growth as it does in all these systems as shown by pH monitoring and CO_2 supplementation. These systems offer many opportunities for integrating seaweed culture with finfish or shellfish culture to use the seawater more efficiently and to benefit from the seaweed's extractive metabolism in removing nitrogenous compounds from the animal culture effluent. With appropriate integration with fossil fueled power plants, seaweeds may also contribute to carbon dioxide scrubbing from the flue gases. The effectiveness of such integration will likely depend on seasonal effects on growth in outdoor systems. One of the larger challenges in such integration will likely be obtaining rapid feedback on the mineral nutrient demands of the seaweeds. While pH provides rapid and convenient feedback on carbon dioxide uptake, methods for similarly rapid analysis (i.e., sufficiently rapid to supplement the limiting nutrient[s] within a few hours) of the numerous mineral nutrients that might limit seaweed growth in a closed or semi-closed system are either non-existent or very expensive.

Acknowledgements

Financial support and facilities access for this research was provided by NOAA/NMFS, Washington Sea Grant, Washington Technology Center, Söliv International Corp., and the University of Washington. The research benefited greatly from the ingenuity and sustained effort, often under cold and wet conditions from the sustained efforts of E.W.("Pete") Duffield, E.C.S.("Ellie") Duffield, M. Costanza, K. Cooley, M. Brockington, D. Boratyn, C.T. Jensen, and E. Mahler.

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Bull. Fish. Res. Agen. Supplement No. 1, 101-109, 2004

Economic value of tideland as place for recreational clam digging, a case study at Kajishima Island

Yasuji TAMAKI*

Abstract Clam digging produces large revenue for fishery cooperative associations and produces a large additional income to fishermen. Although many urban residents enjoy clam digging, an evaluation of the economic value has not been carried out until now. Therefore, I estimated the recreational benefits of clam digging by the travel cost method by using the embarkation lists of the ferryboats for recreational clam digging visitors at Kajishima Island, Kira Town. The results totaling 35.65 million yen were calculated as the recreational benefits of Kajishima Island's surroundings of about 6 ha from the clam digging bed. This amount with Manila clam fishing amount of the Kira Fishery Cooperative Association of 38 million yen. It is evident that urban residents gained a large benefit from recreational clam digging. Mitigation by artificial tidal land is necessary for the tidal land lost by land reclamation as a future policy.

Key words: clam digging, recreational benefits, travel cost method

Among various important uses of tideland such as purification of water and nursery of aquatic animals, the function as a place for recreation and tourism should not be disregarded. Clam digging has been traditionally major recreation performed on tideland in Japan. Manila clam is dominant species caught by recreational clam digging. However, the catch of Manila clam in Japan by commercial fisheries has been decreasing recently and recreational clam digging is no longer carried out in many areas depending on only local resources. Many fishermen's cooperative associations are releasing recently seeds of Manila clam along their coast to compensate the decrease, and to continue clam digging habit. Under this situation, estimation of economic value is becoming important though there is no research on the economic value of clam digging except the report by Aoyama *et al.* (1996), which considers the value from the view point of water purification function. In this study, I estimate the economic value of clam digging in Kajishima Island as a case study.

1. Present status of clam digging in Kajishima Island.

Kajishima is located in Mikawa Bay facing to Miyazaki Port, Kira City, Aichi, Japan. The shore line of the Kajishima Island is 1.8 km and the distance between Miyazaki port and Kajishima Island is 1.5 km. The nearest large city is Nagoya City and distance between Miya-zaki Port and Nagoya is 50 km and 1.5 hours by car. The shore where clam digging is possible is about 2/3 of the circumference of the island. An area of the clam digging ground is estimated at about 6 hectares. Most of clam digging grounds of this island are shores which have many stones instead of a tideland of a sandy area. Manila clams live in the sand which occurs between these stones (Fig. 1). Clam

2003年６月12日受理（Received on June 12, 2003)
* National Research Institute of Fisheries Science, Fukuura, Kanazawa, Yokohama 236-8648, Japan

digging in Kajishima Island is administrated by Kira Fishermen's Cooperative Association (KFCA). KFCA operates 14 small trawl net fishing boat as transportation between Miyazaki Port and Kajishima Island during the season. The price of round ticket by boat between Miyazaki port and Kajishima is 1,100 yen and tourist for clam digging in Kajishima Island pay 1,200 yen as a charge for clam digging to KFCA. The passenger on the boat has obligation to fill up the passenger list with their name and address. A net is handed to a tourist as the payment of clam digging charge and a tourist can catch the clam up to the limit of the volume of the net. A net can accommodate about 10kg of clam.

Aside the income of clam digging charge, KFCA collect 8 % of the boat charge, 5 % as catch commission and 3 % as Manila clam seed price from the captains of trawl net fishing boats.

2. Present status of Manila clam fishery and recreational clam digging

Aichi prefecture had recorded the highest catch among prefectures in Japan for 14 years until 2000 except 1995 (Fig.2). The amount of the catch by Manila clam fisheries in Aichi Prefecture decreased after the peak in 1989 (Fig.3). Table 1 shows the number of people who visited shore of prefectures for recreational clam digging from fisheries census in 1998. Nine hundred and ninety five thousand people visited the shore of Kanagawa Prefecture and this was the highest record of visitors in 1998. Less than half of the highest record visited to coast of Aichi Prefecture (424,000). This number attributes to 10 % of total number in Japan. Recreational clam digging in Aichi is supported by fishermen's cooperative associations. There are many fishermen's cooperative associations that have the section for recreational clam digging service. They release 18×10^8 Manila clam seeds (57 % of total released seed in Japan) for stock enhancement and more than 80 % of tourist who came to shore of Aichi for clam digging used the guidance by fishermen's cooperative associations (Table 2).

Table 3 comes from investigation of fishermen's cooperative associations. Table 3 is showing management rules and present status of clam digging controlled by 13 fishermen's cooperative associations in central part of Mikawa Bay, from Gamagouri City to Isshiki Town. The seasons for recreational clam digging and number of visitor differ among fishermen's cooperative associations. The lowest number of visitors is 679, and highest number of visitors is 70,000. The quantity of released seed ranged

Fig. 1. Kajishima-island clam digging spot

Table 1. Number of clam digging visitors

Prefecture Name	Number of clam digging visitors (total)	Number of clam digging visitors who used guidance of fishermens' cooperative associations
Kanagawa	995,000	5,000
Mie	734,800	260,500
Chiba	652,800	480,800
Aichi	424,100	395,200
Ibaraki	396,600	0
Okinawa	178,000	1,600
Hyogo	167,300	138,700
Hiroshima	117,900	26,100
Shizuoka	117,000	74,300
Kagoshima	103,200	0
Wakayama	86,700	82,800
Oita	79,600	21,000
Osaka	64,300	0
Kumamoto	54,700	9,100

Source: Fishery Census (1998)

from 1 to 126 ton. The coefficient of correlation between the number of visitors and the quantity of seed released was high with 0.888. In other words places with many visitors correlate with high seed release. The charge for adult varies from 900 to 1,400 yen. The highest price is 2,300 yen when the ferryboat charge is added. The upper limit of catch allowed to visitor is from 2 to 10kg, and some places are allowed to catch clams without limit.

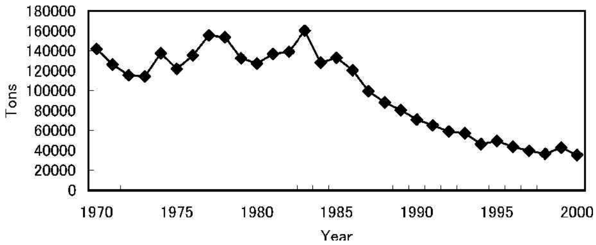

Fig. 2. The change of total catch by Manila clam fishery in Japan Source: Fishery Statistics

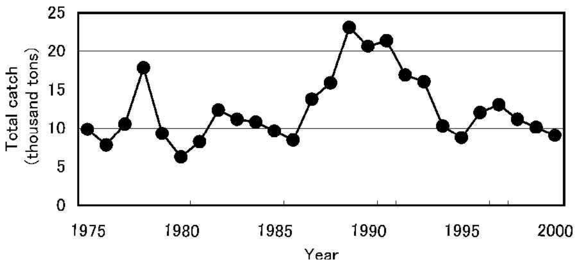

Fig. 3. The change of total catch by Manila clam Fishery in Aichi Source: Fishery Statistics

Table 2. Number of Manila clam seed released

Prefecture Name	1998 (billion)
Aichi	18.08
Mie	2.9
Chiba	2.43
Yamaguchi	1.88
Kumamoto	1.48
Fukuoka	1.24
Oita	1.07
Shizuoka	0.9
Nagasaki	0.68
Miyagi	0.55
Fukushima	0.21
Hiroshima	0.08
Hyogo	0.06
Ehime	0.03
Others	0.09

Source: Fishery Census (1998),

Method

I estimate economic value of recreational clam digging for KFCA and fishermen who carry visitor by fishing boat. I estimate recreational benefits of recreational clam digging for visitors by using travel cost method. The passenger list of boat transportation between Miyazaki Port and Kajishima Island was used in the estimation of number of visitor and specification of address of each visitor. I estimate travel cost (TC) of each visitor by summing up their monetary expenses and time expenditure. In the estimation of travel cost, necessary time and distance were calculated by assuming that each visitor came from their municipality office and they used toll way when the distance exceed 100 km. In the estimation of traffic expenses, rate of fuel consumption is 15 yen a km. Monetary expenses include a traffic expenses, a toll, the price of round ticket by boat between Miyazaki Port and Kajishima and a charge for clam digging to KFCA. In the estimation of time expenses, opportunity expenses of round trip and clam digging were calculated by using of average wages rate. I estimate visit rate (V) by using each population of municipality and visitor's number of each municipality. I estimate relational expression between TC and V. I estimate visitor's number of each municipality by using this relational expression and TC. I sum up by 500 yen to the TC of each municipality until visit rate down to 0. I sum up visitor's number of each municipality by an additional expenses (TC'). I calculate acceptable rate (V') of each TC'. I estimate relational expression between TC' and V'. I can find the visitor's average recreational benefit by integrating this equation.

Table 3. Aichi clam digging enforcement situation comparison

	Period for clam digging in 2002	Charge for adult (yen/indiv./day)	Limitation of catch for adult (kg/indiv.)	Charge for child (yen/indiv./day)	Limitation of catch for child (kg/indiv.)	Ferryboat charge (yen/indiv.)	Amount of Manila clam seed released (ton/year)	Accommodation number of a parking	Charge for parking (yen/car)	Number of visitor a year (2000 or 2001)
A	2/28～6/27	1,200	5	600	2.5	-	1.0	150	0	679
B	2/28～6/27	2,000	5	1,000	2.5	800	3.2	-	-	1,096
C	3/28～6/16	900	2	900	2	-	60.8	400	400～500	25,000
D	3/1～5/30	1,200	4	700	2	-	13.0	80	0	2,602
E	3/17～7/29	1,400	4	700	2	-	126.0	2,000	0	70,000
F	3/17～7/29	2,100	4	1,400	2	700	18.0	-	-	10,000
G	3/1～7/28	1,400	4	700	2	-	28.0	300	0	9,482
H	2/28～6/25	1,400	10	700	5	-	46.0	500	0	3,300
I	2/28～6/23	2,300	10	1,500	5	1,100	50.0	-	-	11,000
J	3/14～6/16	1,300	unlimited	700	unlimited	-	60.3	500	0	15,263
K	3/1～6/29	1,000	unlimited	500	unlimited	-	8.5	1,000	0	10,335
L	4/beg.～6/beg.	1,000	unlimited	500	unlimited	-	2.0	1,000	0	2,000
M	3/16～5/31	1,200	5	600	3	1,600	40.0	-	-	4,700

Source: Information from fishery cooperative associations

Results

1. Economic value of recreational clam digging for KFCA

The fiscal year of the KFCA begins in May and is over in April, the season of clam digging is divided into two financial years. The income of the KFCA in 2001 was 14.66 million yen from the recreational clam digging visitors and 1.43 million yen from the fees from the ferryboat fishermen. As for the outgoing of the KFCA, the Manila clam seed price for release was the biggest expenditure with 6.25 million yen, and wages were 490 thousand yen, and the enterprise profit that subtracted the outgoings from the incomings was 9.36 million yen (KFCA, 2001)(Table 4). In addition, a subsidy of about 3 million yen is expended to the seed price from Kira Town.

Table 4. An enterprise effect by recreational clam digging in the Kira Fishermens' Cooperative Association from 1 May 2000 to 30 April 2001

(units: million yen)

Income	
The income from recreational digging	14.66
The income from the ferryboat fishermen	1.44
Expenses	
Seed release expenses	6.25
Wages for watchmen	0.49
Profit	9.36

Sources: KFCA, 2001 and hearing investigation from KFCA

2. Economic value of recreational clam digging for fishermen who ferry visitor by fishing boat

Clam digging was carried out for 32 days from 24 March to 24 June in 2001 in Kajishima Island. The number of embarkation persons last year (2001) was 7,276, there was about 8 million yen income for fishermen from adult recreational diggers. Fishermen paid 8 % of income to the KFCA. And it was 525,971 yen income for a vessel a season.

3. Economic evaluation of the recreational benefits for visitor from recreational clam digging in Kajishima Island by travel cost method

Clam digging visitors came from 17 prefectures, but 88 % were from Aichi Prefecture (Table 5). Nagano and Gifu accounted for around 4 %. In Aichi Prefecture, Kira Town occupy 11 %, and Okazaki City 10 %, Nagoya

Table 5. Clam digging visitors from various prefectures

Prefecture	People	Percent
Total	7,276	100.0
Aichi	6,432	88.4
Nagano	312	4.3
Gifu	261	3.6
Mie	62	0.9
Shizuoka	50	0.7
Yamanashi	28	0.4
Kanagawa	22	0.3
Ishikawa	15	0.2
Tokyo	14	0.2
Osaka	5	0.1
Saitama	4	0.1
Niigata	4	0.1
Shiga	3	0.0
Fukui	3	0.0
Kyoto	2	0.0
Gunma	2	0.0
Chiba	2	0.0
Unknown	55	0.8

Source: Embarkation list of 2001

City Minato Ku 7 %, Nishio City 7 %, Gamagoori City 5 %, Toyota City 5 % rank high (Table 6).

(1) Monetary expenses

I include the clam digging charge of 1,200 yen, ferryboat charge of 1,100 yen and traffic expenses for monetary expenses. I could specify 7,221 addresses that came from 187 municipalities from the embarkation list. The measurement of distance from each municipality's government office to the Miyazaki Fishing Port was taken from the motoring distance with commercial map software. The measurement of money of road toll uses the map software, too. Because I used adult charge, it is calculated to raise it slightly because there were some children diggers. In addition, because for the embarkation person from Kira Town, as many visitors from Miyazaki District traveled on foot no charge for transportation was assumed and I assumed that visitors from other districts came by car and used the distance from a Kira Town Government Office to Miyazaki Fishing Port. Family groups were assumed to travel together and the expenses averaged for the number of people and, about all the municipalities, calculated the amount of average one person and weighted average of them and calculated the average transportation expenses for each municipality. By the travel cost method, it does not exclude a person having other purpose of trip, and thus it may overestimate. However, the outskirt of Kajishima Island and Miyazaki Fishing Port do not have any other big recreation facilities elsewhere. I judged the purpose of embarkation visitors only for clam digging so that it was necessary

Table 6. Clam digging visitors by municipality of Aichi Prefecture

Municipality	People	Percent
Total for Aichi Prefecture	6,432	100.0
Kira Town	710	11.0
Okazaki City	645	10.0
Nagoya City Minato Ku	459	7.1
Nishio City	453	7.0
Gamagoori City	342	5.3
Toyota City	310	4.8
Anzyou City	278	4.3
Hekinan City	177	2.8
Tiryu City	167	2.6
Hazu Town	163	2.5
Handa City	152	2.4
Kasugai City	147	2.3
Kariya City	144	2.2
Nagoya City Midori Ku	132	2.1
Toyohashi City	126	2.0
Nagoya City Nakagawa Ku	119	1.9
Nagoya City Minami Ku	99	1.5
Kisogawa Town	87	1.4
Toyoake City	84	1.3
Ohbu City	80	1.2
Nagoya City Kita Ku	72	1.1
Tohkai City	69	1.1
Kohta Town	64	1.0
Isshiki Town	63	1.0
Chita City	60	0.9
Ichinomiya City	59	0.9
Nagoya City Tenpaku Ku	59	0.9
Taketoyo Town	57	0.9
Nagoya City Moriyama Ku	56	0.9
NagoyaCity Mizuho Ku	56	0.9
Komaki City	53	0.8
Miyoshi Town	51	0.8
Others of Nagoya City	266	4.1
Others of Aichi Prefecture	573	8.9

Source: Embarkation list of 2001

for visitors who gathered clam at low tide to take home live clams with them because most visitors return home after clam digging immediately.

(2) Time expenses

I measured tour time to the Miyazaki Fishing Port from the municipality office of residence of each embarkation person by map software. Furthermore, I applied tour times to have made use of an expressway in case that they exceed 100 km one way. The necessary time for clam digging is 3 hours and 30 minutes. I multiplied for the expenses rate in an opportunity of a tour time for the total in the aforementioned necessary times. I used an average wages rate R in the following equation as an expenses rate in an opportunity of a tour time (Fujimoto, 1996).

$$R=(w\times(1-T)/Hw)\times(E/M) \quad (1)$$

Where w is average monthly cash earnings for regular employee by prefecture (establishments with 30 employees or more) (Statistics and Information Department, Ministers' Secretariat, Ministry of Health, Labor and Welfare, Japan, 2002). The term T is tax ratio against each prefecture inhabitant of a prefecture income (Government Cabinet Prefecture, 1998, Local Public Finance Investigation Research Society, 1999 and National Tax Agency, 1999). The term Hw is average monthly hours worked by a regular employee at prefecture (establishments with 30 employees or more) (Statistics and Information Depart-ment, Ministers' Secretariat, Ministry of Health, Labor and Welfare, Japan, 2002). The term E is the number of persons who work in one household with each prefecture office location city (Statistics Bureau, Ministry of Public Management, Home Affairs, Posts and Telecommunications, Japan, 2001)

The term M is the number of persons in one household with each prefecture office location city (Statistics bureau Ministry of Public management, Home Affairs, Posts and telecommunications, Japan, 2001)

I summed up aforementioned monetary expenses and time expenses and calculated the travel cost (TC) for one person for each municipality.

(3) Visit rate (V)

I estimate V (percentage) from the following formula. Divide visitor's number of each municipality by each population of municipality (thousand persons).

(4) Calculation of equation of V and TC

Because I applied function style by the least-squares method in respect of scattering of V−TC, applying to it indeed for natural logarithm equation was good. The relation between V and TC can be expressed by the following equation. (Fig. 4)

$$V=-181.496\log TC+1808.978 \quad (2)$$

The P value was 0.000113 and meaningful though the decision coefficient is low with 0.08.

(5) Estimation of visitor number of each municipality.

I calculated the estimated V of aforementioned equation based on TC for each municipality. Then I found the estimation number of visitor for each municipality.

(6) Measurement of the additional expenses (TC') and accept rate (V')

I added additional expenses until an estimation visitor number became 0 by 500 yen for TC every municipality. Because I applied function

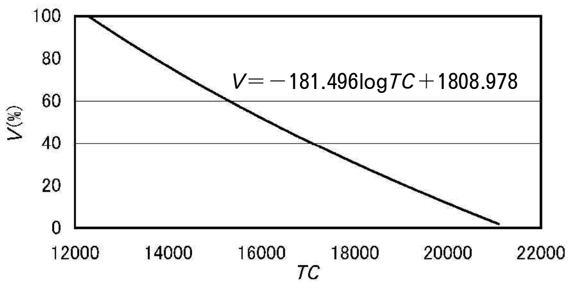

Fig.4. The relation between V and TC

style by the least-squares method in respect of scattering of $V'-TC'$, applying to it indeed for natural logarithm equation was good. Relation between the additional expenses (TC') and the accept rate (V') can be expressed by the following equation.(Table 7 and Fig.5)

$$V'=-0.33809\log TC'+3.239559 \quad (3)$$

The value P was 1.21×10^{-19} and meaningful with a 0.954 decision coefficient.

(7) Calculation of the average *WTP* (willingness to pay)

If I decide demand curve of equation (3), integrate it, I can calculate the average *WTP*.

$$WTP=\int_a^b(-0.33809\log TC'+3.239559)\,dTC'$$

a: 0

b: 14,077 yen (accepting 1 percent)

The average *WTP* for a person was 4,900 yen by this equation.

(8)Estimation of the recreation benefits

Average *WTP* 4,900 yen × 7,276 person = 35,650,000 yen

Recreation benefits of visitor at Kajishima Island estimated 35.65 million yen.

Discussion

Because of the enterprise, general profit of the KFCA in 2001 was about 42 million yen, and the profit from recreational clam digging accounts for 22 % of general profit, and its benefit in the KFCA is high. Clam digging is carried out in a lot of fishermen's cooperative associations in Mikawa Bay, and an enterprise profit of a fishermen's cooperative association plays an important role.

At Kira Town, fishermen carry clam digging visitors after their trawling operation, so the pickup income is added to a fishing income. Because the unloading of a small trawl net of 2001 was about an average of 7.5 million yen, the pickup income comprises around 7 %. However, as for the fishery income, pickup income (525,971 yen) amount to the 23 % for 2.3 million yen by unloading 7.5 million yen. It became clear that clam digging brings large additional income to fishermen who carry visitors.

Recreational benefits of clam digging for

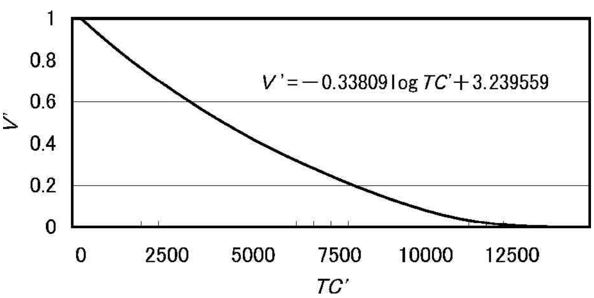

Fig.5. The relation between V' and TC'

Table 7. Measurement of the additional expenses (TC') and accept rate (V')

TC' (Additional expenses (yen/indiv.))	Estimated number of accepted person of all municipalities	V' (Accept rate)
0	13,530	1.0
500	12,550	0.92759
1,000	11,616	0.85856
1,500	10,733	0.79332
2,000	9,906	0.73216
2,500	9,114	0.67361
3,000	8,359	0.61779
3,500	7,638	0.56454
4,000	6,958	0.51426
4,500	6,309	0.46633
5,000	5,694	0.42086
5,500	5,122	0.37856
6,000	4,580	0.33853
6,500	4,069	0.30073
7,000	3,576	0.26433
7,500	3,102	0.22925
8,000	2,642	0.19528
8,500	2,222	0.16422
9,000	1,821	0.13457
9,500	1,455	0.10751
10,000	1,103	0.08152
10,500	802	0.05927
11,000	561	0.04147
11,500	368	0.02718
12,000	226	0.01667
12,500	123	0.00909
13,000	54	0.00397
13,500	23	0.00172
14,000	9	0.00070
14,500	2	0.00016

visitors are estimated 35.65 million yen at Kajishima Island. This amount of money is almost equal to 38 million yen of quantity of Manila clam fishery of 151 tons a year in the KFCA. It is obvious that urban residents give a high evaluation against recreational benefits of clam digging.

A lot of city inhabitants enjoy clam digging as natural scene or object which adds to poetic charm to the season, but, on the other hand, a tendency to decrease Manila clam population, and an expense of seed discharge rises, too. In addition, landfill goes ahead through a tideland of the coast, and fishing grounds of clam digging are being lost. For example, large quantities of Manila clam died by high water temperature and an outbreak of blue tide in Mikawa Bay in 1994. And a red tide by Heterocapsa occurred on the coast that reached Gamagoori City from Issiki-town in summer, 2000, and Manila clam died in large quantities (Sonda and Kimura ., 2001). Clam digging was canceled due to shellfish poisoning which occurred in spring, 2001, until a declaration of safety came out on 19 March, and, it is a grave concern.

I estimated the recreational benefits of clam digging at 35.65 million yen as the recreation benefits of about 6 hectares Kajishima Islands' clam digging ground. It became clear that the recreational benefits of clam digging for city inhabitants were equivalent to the fishery amount of money and the scale.

Maintenance of the water quality is important, but in fact the water purification ability of a tideland where clam digging is possible is the most effective for the improvement of the water quality, and enhancement of artificial tidal land is necessary for the tidal land lost by land reclamation as a future policy so that we can continue clam digging in future. I would like to do such an economic assessment of tideland to judge whether the budget expenditure is right or not when a country and a local self-governing body enforces mitigation and supports clam digging. In addition, producer of the artificial tideland must not make the new environmental disruption by collection of sand to make the tideland.

The number of people digging clam according to prefecture by a fishery census, Kanagawa Prefecture was the first place, but most people are the people who came to the Park of the sea which is an artificial tideland made in reclaimed land of Kanazawa-ku, Yokohama City, Kanagawa Prefecture. But there is not management by fishermen's cooperative associations, and indiscriminate gathering becomes a problem because there is not fishery right in the Park of the sea (Kudou, 2000). It is thought that the role of management by the fishermen's cooperative associations in a clam digging fishing ground is very important.

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Bull. Fish. Res. Agen. Supplement No. 1, 111-121, 2004

Large-scale restoration of tidal flats and shallows to suppress the development of oxygen deficient water masses in Mikawa Bay

Teruaki SUZUKI*

Abstract Development of hypoxia has been confirmed in the inner-part of almost every major bay of Japan on the Pacific coast from Tokyo southward. Mikawa Bay, where Japan's most serious hypoxia occurs, is used to this report to present the effects and causes of hypoxia, such as impact upon fisheries, historical development and nutrient budget between sediment and water. Although hypoxia basically results from an increase in nutrient load input, intense reclamation in Mikawa Bay of about 1,200 ha of shallows in the 1970s, including tidal flats, has drastically accelerated a deficiency in dissolved oxygen. This is mostly due to losses in the rich filter-feeding macrobenthic community that largely control the high water-purification capacity of those areas. Currently, oxygen deficient water masses in Mikawa Bay are large enough to strip the precious water purification capacity of the remaining shallows by killing the remaining filter feeders. Consequently, the considerable shallows have turned from being a purifier of water quality to a source of excess nutrients, thus sending the Bay into a spiral of deterioration. In order to break this vicious cycle, the dissolved oxygen deficiency of the Bay must be contained to the extent that the purification capacity of the shallows can be brought into full play. To this end, the first thing to do is to restore the tidal flats and the shallows having the effective depth so designed as not to be affected by oxygen deficient water masses, over an extensive area. This may be a more urgent imperative than reducing the nutrient load input. Since 1998 to 2001, about 350 ha of artificial shallows, including tidal flats, have been restored in Mikawa Bay using sand dredged from the Nakayama sea channel. Recovery of abundant benthic organisms, such as bivalves, has been confirmed already by monitoring. Additional tidal flat restoration is now in progress.

Key words: hypoxia, tidal flat, restoration, *Ruditapes philippinarum*

Effects of a dissolved oxygen deficiency on fisheries in Mikawa Bay

Mikawa Bay is a typical, partially mixed estuary located in the central part of Japan as shown in Fig. 1. The Bay consists of two inlets: The northwest part is Chita Bay into which the River Yahagi-gawa flows. The eastern part is Atsumi Bay into which the River Toyo-gawa flows. In its entirety, Mikawa Bay measures about 600 km^2 and is very shallow with an average depth of 10m.

Oxygen deficient water masses occur in Mikawa Bay on a large scale as seen in the example shown in Fig. 2 (Ishida and Hara, 1996) since about 1970. Oxygen deficiencies can last as long as four months, or more, from mid-June to mid-October.

Mikawa Bay was one of the best fishing grounds for commercial crustaceans (such as

2003年６月12日受理（Received on June 12, 2003)
* Aichi Fisheries Research Institute, 97, Wakamiya, Miya-cho, Gamagori, Japan, 443-0021

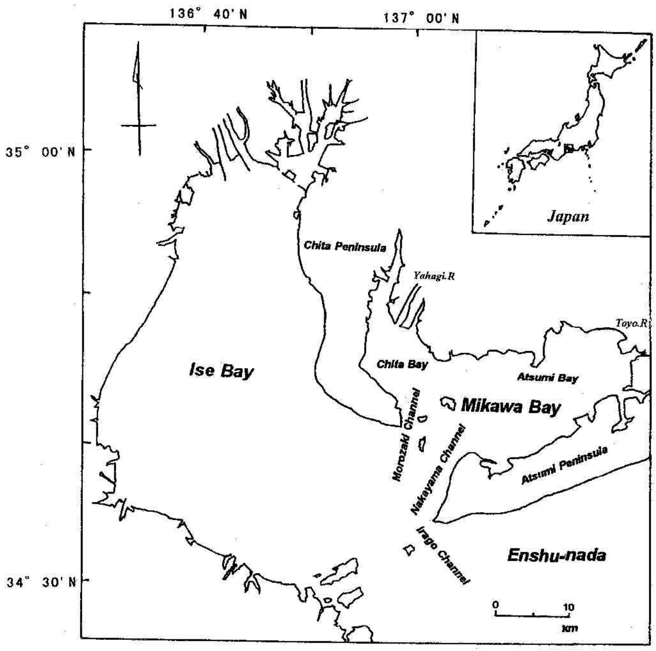

Fig. 1. Map of Mikawa Bay

kuruma-prawns, swimming-crabs), bivalves (such as short-necked clams, cockles) and benthic fishes (such as stone flounders) in Japan. For example, the catch of the short-necked clam (*Ruditapes philippinarum*) has been the best in Japan until today, contributing the largest share of about 36 % to the total yield. However, the total catch in Aichi Prefecture decreased from 20,000 ton year^{-1} to 11,000 ton year^{-1} from the latter half of the 1960s to 2001.

Since 1970, when dissolved oxygen deficiencies began to develop, the major fishing grounds of small-trawl fisheries of these species shifted from the middle of the bay towards the mouth. At present, the Mikawa Bay trawl fishery yield is rapidly declining.

Effects of dissolved oxygen deficiencies on the Mikawa Bay nutrient budget

A deficiency of dissolved oxygen not only affects the structure of a benthic community, but also its functionality. We have studied the temporal development of waters deficient in dissolved oxygen and the resulting benthic community changes (Suzuki *et al.*, 1998a). We have also studied how the nitrogen balance between the benthic sediment and seawater is affected, using a benthic ecosystem model (Suzuki *et al.*, 1998b) that was developed to quantify the nitrogen cycle of tidal flats in Mikawa Bay (Suzuki *et al.*, 1997). Those results are outlined below.

Fig. 3 shows the temporal changes in water temperature, salinity, and dissolved oxygen saturation percentage immediately above the bottom sediment in shallow areas (4 m depth expressed by chart) that were recorded in the inner part of Mikawa Bay, from June to the end of July 1996, and compared to changes in the benthic community (bacteria, benthic micro algae, meiobenthos, and macrobenthos).

A deficiency in dissolved oxygen was observed on 25-26 June, 28-29 June, and 10 July , but each time the occurrence was brief and the saturation level stayed at about 50 % until at least 20 July. Then, a deficiency in dissolved oxygen lasted through the period from July 20-

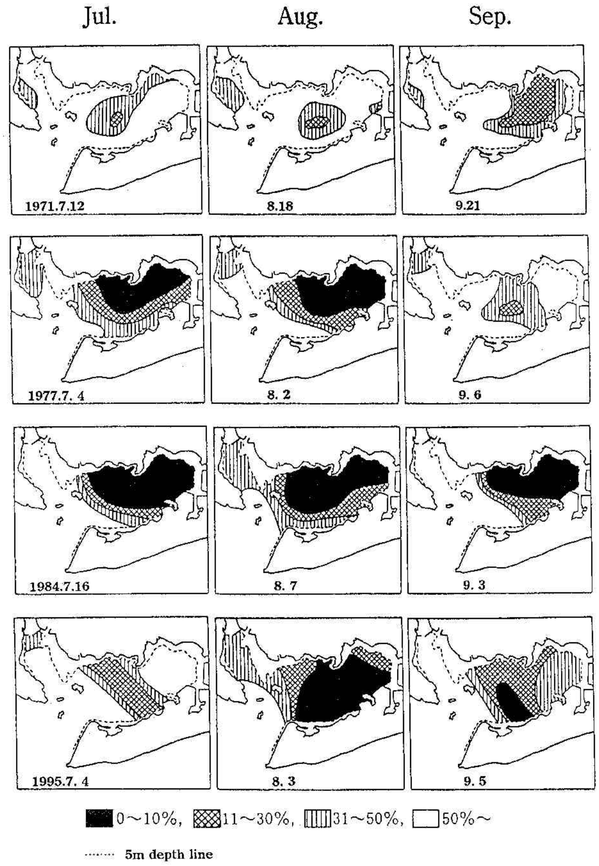

Fig. 2. Example of the distribution of dissolved oxygen deficient water masses in Mikawa Bay. Four legends denote the level of dissolved oxygen saturation percentage

29 and the frequency of anoxic conditions increased.

Different species reacted in different ways to low oxygen concentrations according to their tolerance limits, but eventually on July 29 the biomass of every species dropped sharply.
Fig. 4 shows a time-series chart of the nutrient flux between seawater and sediment calculated by a benthic ecosystem model. On the vertical axis, the flux from seawater to sediment is indicated as negative quantities.

Before 16 July, particulate organic nitrogen (PON) was being removed from the water at -561 to -962 mgN m^{-2} day^{-1} (-785 mgN m^{-2} day^{-1}, on average). Dissolved inorganic nitrogen (DIN) was eluting at 159 to 757 mgN m^{-2} day^{-1} (535 mgN m^{-2} day^{-1}, on average). On balance, Total nitrogen (TN) was removed from the water at -43 to -401 mgN m^{-2} day^{-1} (-250 mgN m^{-2} day^{-1}, on average). Then, from 16 to 22 July, both the PON removal rate and the DIN elution rate dropped rapidly, and on 29 July PON removal had almost ceased. Thus, after 22 July, the TN budget turned from negative to positive to about 240 mgN m^{-2} day^{-1}.

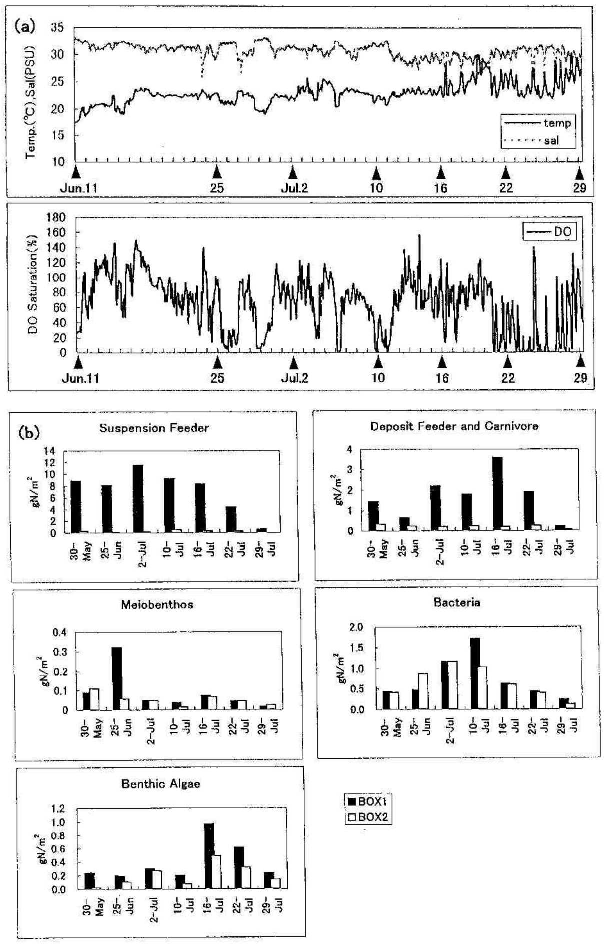

Fig. 3. Time-series changes in water temperature, salinity and degree of dissolved oxygen saturation percentage immediately above the bottom sediment in shallow areas of Mikawa Bay and changes in the benthic community (bacteria, benthic micro algae, meiobenthos, and macrobenthos) from June to end of July in 1996 (Suzuki *et al.*, 1998a)

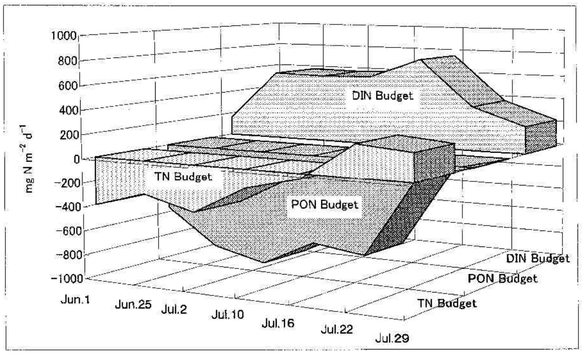

Fig. 4. Change of the nitrogen budget between sea water and sediment on the shallow area of the inner Mikawa Bay from 1 June to 29 July, 1996 (Suzuki *et al.*,1998b)

The analytical results from the benthic ecosystem model indicate that the shallows usually have the capacity to reduce particulate organic matter at a higher rate of efficiency than the rate of elution of inorganic nutrient salts. However, this so-called "water purification capability" functions only if ambient oxygen conditions are normal. If a large oxygen-deficient water mass develops in the offshore sea bottom to a scale that can affect the shallows under certain wind and tide conditions, the situation changes completely.

First, the macrobenthic biomass of suspension-feeders decreases, resulting in a lower rate of removal of particulate organic matter from the water. Then, the rate of removal of organic sediment matter by macrobenthic deposit-feeders, which balances with the rate of sedimentation of PON, also drops. Subsequent deterioration in water transparency near the sea bottom hinders the growth of benthic microalgae and seaweeds, which slows down the absorption rate of inorganic nutrient salts. Then, the amount of oxygen generated by photosynthesis also falls, accelerating the development of the anaerobic condition near the sea bottom.

Circulation of nutrient salts within the bottom sediment becomes heavily dependent on bacteria, although this system is also hindered by a reduction in bacterial biomass. Although this means a reduction in nutrient elution from sediment to seawater, the TN budget of PON and DIN shifts from a sink (−) to a source (+). Consequently, the shallows that were a highly efficient water column purifier become a source of nutrients, and this further exacerbates the dissolved oxygen deficiency.

It is estimated that benthic organisms usually abundant in shallow sea areas above 5 m in depth are wiped out by the deficiency of dissolved oxygen in about one third of the total shallow area under the worst circumstances. This results in nitrogen elution of 11 tons day^{-1}, which amounts to about 27 % of the nutrient load input to Mikawa Bay. This means that countermeasures to reduce the nutrient input, such as construction of sewage treatment plants, would make little difference unless the hypoxic condition is improved first.

Historic record and causes of dissolved oxygen deficiencies

An oxygen deficient water mass is formed when oxygen demand exceeds oxygen supply. The main cause of high oxygen demand is the increase in the amount of particulate organic matter in the water or organic sediments on the bottom.

Fig. 5 shows the transition in transparency and the nitrogen load through time. Transparency rapidly decreased from 1955 to 1970, corresponding to annual fluctuations in the nitrogen load. During that period, the nitrogen load doubled and phosphorus tripled. However, from about 1970 to 1983 as shown in Fig. 6, red tides became a notable feature after that period. The development of dissolved oxygen deficiencies also increased steeply from about 1970. Red tides and associated hypoxia rarely occurred before 1970.

Thus, historically, eutrophication in Mikawa Bay can be divided into Phase I (1955-1970) in which transparency decreased considerably as a result of the increased nutrient load; and Phase II (1970 and after) in which fisheries were severely damaged by red tides and oxygen-deficient water masses. The question now is, apart from the increase in nutrient inputs, what caused the intensification of eutrophication between Phase I and Phase II?

One likely cause is the intense reclamation throughout the extensive shallow areas. During the 1970s in Atsumi Bay, in the eastern-part of Mikawa Bay, about 1,200 ha of shallows, including tidal flats, were reclaimed to prepare for constructing a harbor (see Fig. 7). The appearance of red tides, accompanied by oxygen deficient water masses, coincides exactly with that reclamation of the shallows. This fact is very important in regard to hypoxia in the estuary. Tidal flats and shallows have a high water-purification capability due to filtering by macrobenthic suspension-feeders. Incidentally, benthic suspension feeders (mainly bivalves) living on the Isshiki tidal flat of Mikawa Bay, are estimated to filter seawater at a rate of 3.4 (Aoyama and Suzuki, 1997) to 5.0 m^3 m^{-2} day^{-1} (Sasaki, 1994). From the reclaimed 1,200 ha, this totals to about 500 m^3 sec^{-1}. This value is calculated by assuming that the standing stock of the benthic suspension feeders in the reclaimed area in 1970s is equal to that of the present Isshiki tidal flat area. However, it is possible that this value is underestimated because the reclaimed area was the richest clam fishing ground in Mikawa Bay. The standing stock of short-necked clams in the reclaimed area was estimated at 3.4 times as much as that in the present Isshiki tidal flat, according to the past catch statistics of Aichi Prefecture. Using this revised value, the reclaimed 1,200 ha of shallows are estimated to filter seawater at a rate of 1,700 m^3 sec^{-1}. Since the rate of exchange of seawater at the mouth of Mikawa Bay is 1,169 m^3 sec^{-1} (Unoki, 2000) to 2,600 m^3 sec^{-1} in the summer (Sasaki, 1989), the lost seawater filtration rate equals 19 % to 43 % of the exchange rate of seawater in the case of 500 m^3 sec^{-1} and 65 % to 145 % in the case of 1,700m^3sec^{-1}.

It seems likely that the reduced capability of seawater filtration drastically reduced the capacity to remove suspended organic matter from the water and consequently, accelerated the development of water masses with a dissolved oxygen deficiency, followed by severe red tides.

There is another possibility besides intensive reclamation. It is the development of water resources in the catchment basin. In Mikawa Bay, the River Toyo-gawa is the most important river in regard to density flow and seawater exchange of the Bay.

In 1968, Toyo-gawa canal was completed, which diverts about 20 % (in an average year) to 40 % (in a dry year) of the total flow of the River Toyo-gawa. The chief purpose of the canal is irrigation of farmlands on the Atsumi Peninsula, for which 72 % of the diversion is used. There is speculation that this large reduction in the river flow, due to the canal diversion, reduces the density flow in the bay and exchange of seawater proportionally by 20 % to 40 %. However, this subject needs further study, since a lower estimation of the decline rate of exchange of seawater has been made in an analysis using a numerical model (Suzuki *et al.*, 1986). Nevertheless, it is certain that the hindrance to seawater exchange slows the ejection of suspended organic matter to the exterior of the Bay; and reduces the supply of dissolved oxygen from the mouth of the Bay.

We surmise that the reduction in the filtration rate of seawater, caused by reclamation of the shallows around 1970s, was what mainly accelerated the occurrence of red tides and dissolved oxygen deficient water masses. As mentioned above, Oxygen-deficient water masses in Mikawa Bay have become large enough to strip the precious water purification capability of the remaining shallows. Unfortunately, the shallows have turned from being a water purifier to a source of nutrients sending the Bay into a spiral of deterioration. This phenomenon, triggered by hypoxia, is very similar to the catastrophic degradation of lake ecosystems caused by several external forces.

Takamura (2002) reports in order to maintain a healthy lake ecosystem, it is necessary to distinguish between acceptable change and changes to avoid. The most important factor affecting this distinction is the concept of "re-

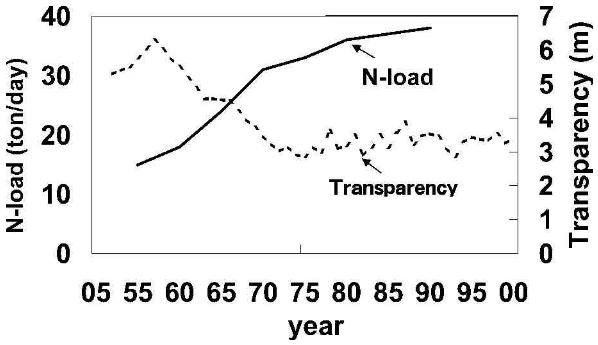

Fig. 5. Transparency and nitrogen load in Mikawa Bay

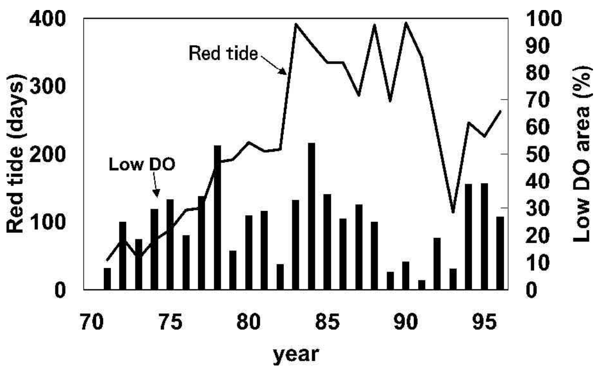

Fig. 6. Total number of days of red tides observed and the areal proportion of oxygen deficient water with less than 30 % saturation in Mikawa Bay

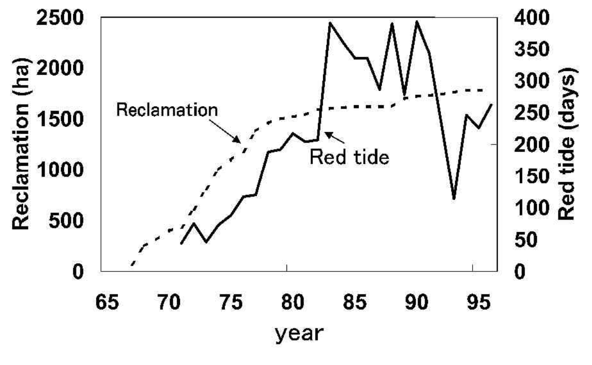

Fig. 7. Total number of days red tides observed and the area of reclaimed land in Mikawa Bay

silience". Takamura (2002) defines lake ecosystem "resilience" as the ability to sustain certain structures and functions of the lake ecosystem against several external forces, and changes that can damage this resilience (catastrophic change) must be avoided.

There are reports in the literature that, through their feeding activities, the macrobenthic community controls the structure of the planktonic community and nutrient concentrations of seawater. They also exert a strong influence on nutrient circulation in the bay (Cloern, 1982; Cohen *et al.*, 1984; Carlson *et al.*, 1984). Thus, in Mikawa Bay, it may be said that abundant bivalves that lived in the reclaimed area provided the Bay with its "resilience". Ironically, the drastic environmental deterioration caused by intensive reclamation projects in Mikawa Bay proved the importance of the benthic community in providing that resilience function.

Restoration of tidal flats and shallows

In order to break the vicious cycle of eutrophication, the dissolved oxygen deficiency of water masses in Mikawa Bay must be contained to the extent that the purification capability of the shallows can be restored to a sufficient level. To this end, the first thing to do is to restore tidal flats and shallows having the effective depth so designed as not to be affected by oxygen deficient water masses, over an extensive area. This may be a more urgent imperative than reducing the nutrient load input.

The Aichi Prefectural Federation of Fisheries Co-operative Associations (APFFCA) organized a technical society to study environmental improvement methods for Mikawa Bay from 1995 to 1997. APFFCA published a proposal titled "The necessity to artificially develop tidal flats and shallows for the improvement of Aichi prefectural sea areas" in 1997. The proposal stated that aggressive restoration of the water purification capability in the Bay would

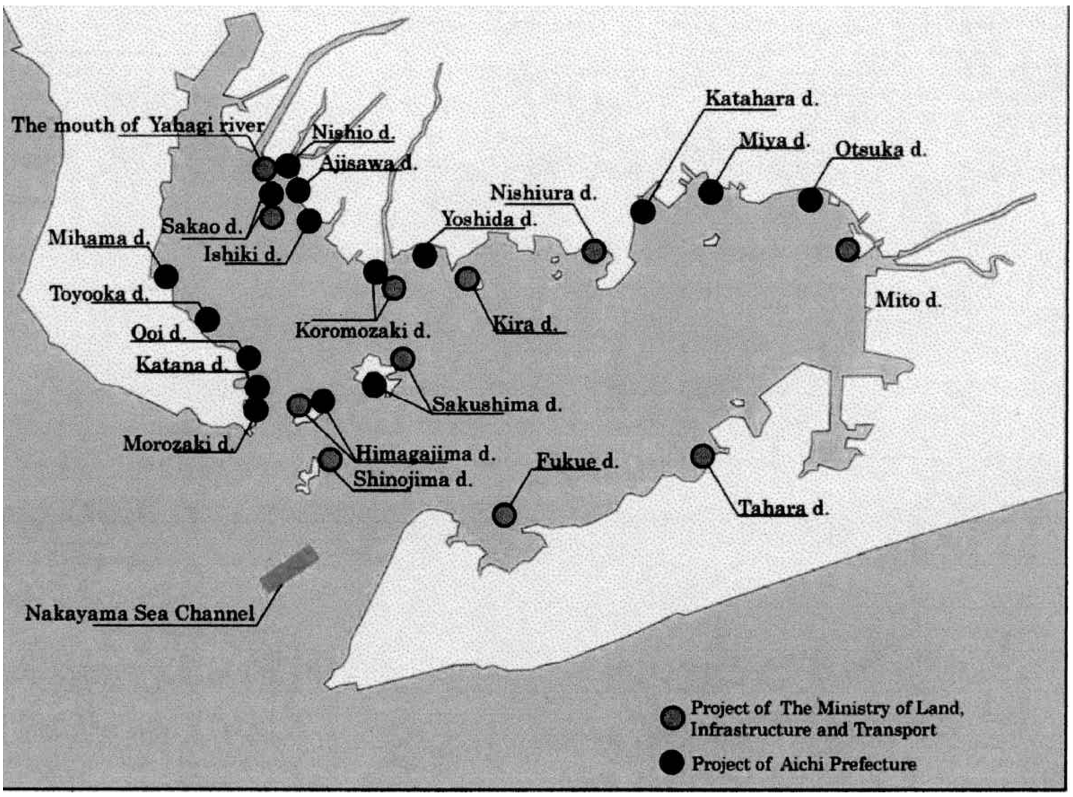

Fig. 8. Locations of the sites restored by 2001

be the most effective way to improve the hypoxic conditions, and then it would be necessary to enhance biological diversity and the productivity of fisheries.

At first, it was thought that realization of this proposal would be very difficult because of the high cost and the large sand volume, given that the APFFCA proposal stated that it would be necessary to restore a large area of over 1,000 ha to significantly suppress hypoxic conditions.

The Nakayama sea channel, located at the entrance of Mikawa Bay, is important to marine traffic accessing the ports in Mikawa Bay. However, the navigation of vessels in the area is limited, because of patchy shoals (the most shallow point is −8.6m) and sunken rocks that interfere with the economical operation of vessels. To improve navigability of the channel, the Ministry of Land, Infrastructure and Transport (MLIT), Chubu Bureau, made the "Nakayama Channel Line improvement plan" in 1980. APFFCA has consistently objected to this plan because this area has been a good fishing ground. As a result of many negotiations between APFFCA and MLIT, it was agreed that all dredged sand (6.2×10^6 m^3) would be used to restore tidal flats and shallows in Mikawa Bay. After this agreement, artificial development of tidal flats and shallows to improve Mikawa Bay accelerated drastically under a joint effort between MLIT and Aichi Prefecture. Fig. 8 shows the locations of the restored sites, in which 11 sites were created by MLIT and 16 sites by Aichi Prefecture. In total, tidal flats and shallows of 350 ha have been restored already using the sand of 3.4×10^6 m^3 from 1998 to 2001. The use of the remaining 2.8×10^6 m^3 is scheduled to restore tidal flats and shallows by 2004.

Effect of the restoration

At some of the restored tidal flats and shallows, monitoring surveys have been conducted on environmental conditions such as water quality, sediment quality, benthic community diversity and abundance of fishery organisms by MLIT and Aichi Prefecture. As the investigations are still going on, we cannot show the results in detail yet. However, parts of the interim results are given in the following:

At the Nishiura site (12 ha) where a tidal flat was created in 1999, many bivalves have been observed-even immediately after construction. In an investigation using a small trawl shell-net (0.59 m width) conducted about 3 years after the tidal flat creation, many megabenthic species, including commercial species, were observed as shown in Table 1. These values are comparable to those of natural tidal flats in Mikawa Bay. Before the creation of the artificial tidal flat, these megabenthic species were not found in this area in summer.

Fig. 9 shows the transition in the numbers of benthic species at the Mito site (10 ha). Initially, the biota consisted mainly of polychaetes (unpublished data by MLIT). As time passed, many additional bivalves appeared such that the biota has become diversified. Improvement of the water purification capacity is expected due to the increase of bivalves because they have a high water filtration capability. At present, the most dominant species, on a wet weigh basis, is *Mactra chinensis* as is likely at the Nishiura site. The particulate organic nitrogen purification capability of this site has been estimated at 136 mgN m^2 day^{-1}, based on the monitoring results of June 2000 (unpublished data by MLIT). This value, estimated 21 months after the artificial creation, is equivalent to about 60% of the nitrogen purification ability of Isshiki tidal flat, which was estimated at 227 mgN m^2 day^{-1} by Aoyama and Suzuki (1997).

At the Isshiki site (26 ha), a lot of juvenile flounders were caught using a standard sledge net (Koshiishi *et al.*, 1999). The most dominant species was the stone flounder (*Platichthys bicolorantus*), which appeared mainly from March to May. A sledge net was towed along three transects on the tidal flat and one transect off the tidal flat at high water. Observed individuals ranged between 3,278-

13,489 inds ha^{-1} (7,156 inds ha^{-1} on average) in March, 2,647-7,037 inds ha^{-1} (5,571 inds ha^{-1} on average) in April and 216-3,315 inds ha^{-1} (1,390 inds ha^{-1} on average) in June, respectively assuming that the efficiency of catch is 0.4-0.7 estimated from the body size (Koshiishi *et al.*, 1999). The values on the restored tidal flat are larger than that of the outside of site. Although the Isshiki site was created in August 2000 and less than one year had passed, these values are almost equal to, or larger than, the density (1,620-8,192 inds ha^{-1}) observed in the natural estuary, of Gamou of Sendai Bay in mid April from 1992 to 1996 (Yamashita *et al.*, 1999). However, there are some sites of artificial shallows where good results that were expected have not been obtained. In summer, at the innerpart of Mikawa Bay, serious hypoxia is frequently observed near the bottom, even in shallows above 4 m in depth. Therefore, the aim of developing a new shallow area should be to avoid hypoxia.

Imao *et al.* (2001) proposed a method to determine the depth necessary to avoid hypoxia at a new artificial shallow area. They proposed that the depth of the artificial shallow should be where the survival rate for the short-necked clam will be over 70 % under the severest hypoxic conditions (hereafter called the "survival depth"). Imao *et al.* (2001) sampled the macrobenthos, water temperature and dissolved oxygen concentration successively in nine coastal areas of Mikawa Bay. Then they estimated each survival depth using a numerical model to forecast the survival rate of short-necked clams against hypoxia (Suzuki *et al.*, 1998c). The results showed that the survival depth is different for each area within a range of D.L. −1.3 m to D.L. −4.0 m. Unfortunately, however, in the actual tidal flat creation, civil engineers, who tend to plan uniformly without reflecting the local hypoxic features, have not yet acknowledged the importance of this method.

We suggest that the depth of the artificial tidal flat creation was slightly lower than the

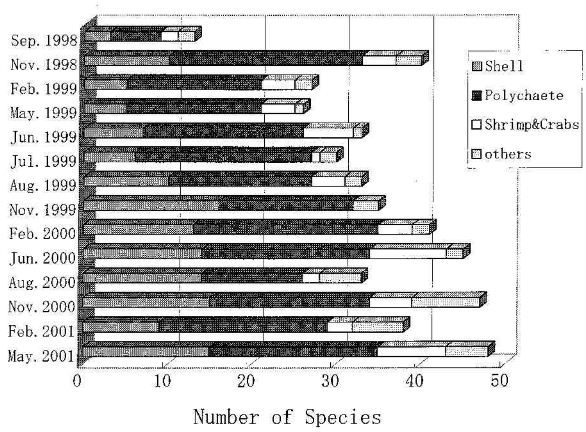

Fig. 9. Numbers of the benthic species at the Mito site (unpublished data by MLIT)

Table 1. Megabenthos caught by a small trawl shell-net (0.59m width) in summer 2002 (21 June; 13 August; 26 August) at Nishiura artificial tidal flat (12 ha) created in June 1999

Species name		inds. m^{-2}	wet. g. m^{-2}
(*Pelecypoda*)			
Mactra chinensis	(bakagai)	2.3-17.2	73-614
Mactra quadrangularis	(shiohukigai)	0.7-22.7	9-194
Ruditapes philippinarum	(asari)	3.4-72.9	21-678
Scapharca subcrenata	(sarubou)	1.2-4.2	6-42
(*Gastropoda*)			
Neverita didyma	(tsumetagai)	0.3-1.5	14-68
Rapana venosa	(akanishi)	0-0.1	0-11
(*Crustacea*)			
Portunus trituberculatus	(gazami)	0-1.0	0-14
Charybdis japonica	(ishigani)	0-0.1	0-1

survival depth at some unsuccessful sites.

There are some problems to solve in addition to the acknowledgement of survival depth. The first is the supply of the sand. As mentioned, we must restore at least 1,200 ha to supress the hypoxic condition in Mikawa Bay effectively, but we can restore only half of 1,200 ha by 2004. After 2004, we shall not be able to get the enough sand to restore. So, we are now studying the alternative materials including the artificial sand such as granulated blast furnace slag, urgently.

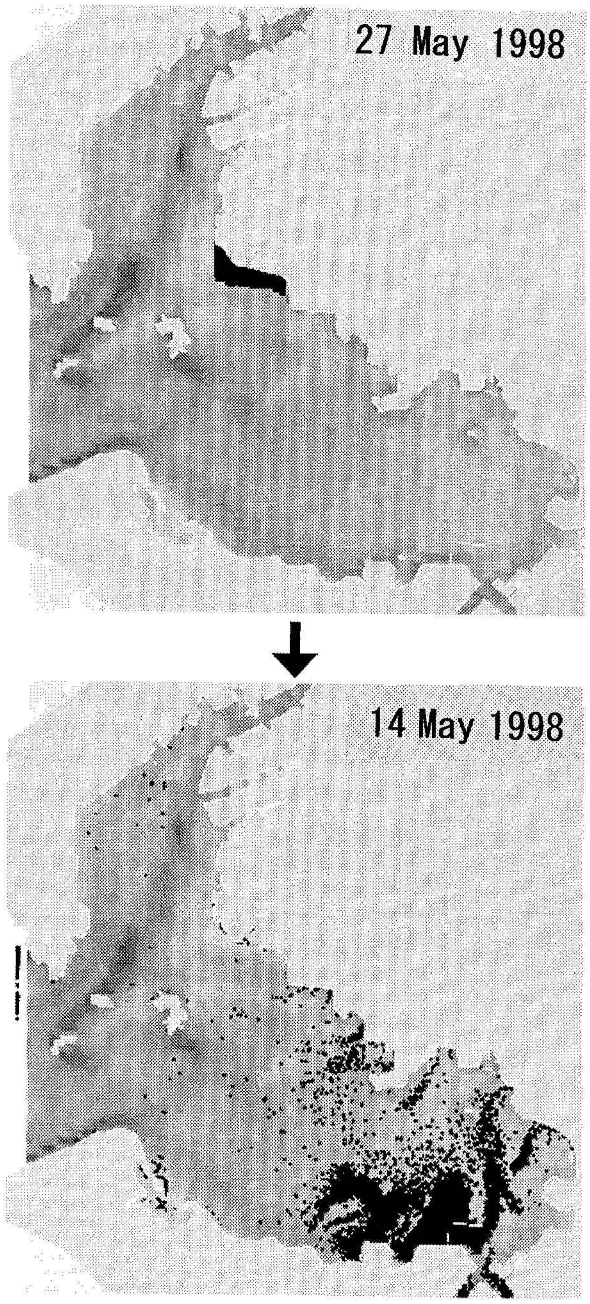

Fig. 10. The example of the final horizontal distribution pattern of pelagic larvae calculated by receptor mode model. Upper figure shows the initial distribution (Suzuki *et al.*, 2002)

The second is the proper selection of the site location. It is important to choose areas that will be the most effective for the recruitment or supply of bivalve's pelagic larvae. To that end, we selected the short-necked clam (*Ruditapes philippinarum*) as a target species, and observed the vertical distribution pattern of the larvae. Then, using a receptor mode model, we are trying to predict trajectories of the pelagic larvae (Suzuki *et al.*, 2002). Receptor mode model is the simulation technique to trace the drift particles in the inverse time mode. Fig. 10 is one of the examples of the calculations (the case calculated at the period from 27 May to 14 May, 1998). Isshiki area where the initial distribution of pelagic larvae was given, is the most important fishing ground of short-necked clam in Mikawa Bay. Pelagic larvae supplied to this area was estimated by receptor mode model, supposing that the drift period is two weeks from the water temperature and the larvae distribute around the mid-depth from the observation. As the model result, it was suggested that the inner part of Atsumi Bay is the most important origin and perhaps it may be the proper site location in future.

Although there are some problems to solve, we will be able to confirm the suppression of hypoxic conditions in future by an increase of the water purification capability, as long as the restoration of tidal flats and shallows progresses smoothly and the existing reclamation plans are abandoned.

Acknowledgements

I wish to thank the staffs of Kinuura Branch of Mikawa Port Construction Office, Chubu Bureau of the Ministry of Land, Infrastructure and Transport for the use of figures and also thank Dr. J. Higano and Dr. T. Seki, National Rsearch Institute of Aquaculture, for their encouragement and helpful suggestions.

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Linking watershed loading and basin-level carrying capacity models to evaluate the effects of land use on primary production and shellfish aquaculture

Mark W. LUCKENBACH* and Harry V. WANG*

Abstract Aquaculture production of hard clams, *Mercenaria mercenaria*, in the lower Chesapeake Bay, Virginia, U.S.A., has increased dramatically within the last decade. In recent years concern has been raised that some growing areas may be approaching the exploitation carrying capacity for clam production. Preliminary calculations indicate that large-scale intensive clam aquaculture may be controlling nutrient and phytoplankton dynamics in this system. To date, carrying capacity models have not been applied to this system, but we are in the process of building models for that purpose. Moreover changing land use in the watersheds surrounding the clam-producing areas raises the need for an improved understanding of how these changes will affect water quality, primary production and shellfish production. We describe an ongoing project linking a watershed-based loading model with a physical transport-based water quality model to simulate primary production and predict carrying capacity for clam aquaculture. Extensive calibration and verification of the water quality model has demonstrated its utility for simulating primary production and water quality parameters in the Chesapeake Bay. In our present efforts, watershed loading models have been developed and tested for predicting both surface and groundwater inputs into the coastal waters. We are currently coupling the water quality and watershed loading models, and developing clam physiology and population-level sub-models. Also, under development is a sediment deposition/resuspension sub-model. Each of these components will be linked to estimate exploitation carrying capacity for clam production in this system. Our goal is to use the coupled models to predict how varying land use scenarios impact water quality, primary production and shellfish carrying capacity of coastal waters.

Key words: *Mercenaria mercenaria*, aquaculture, carrying capacity, water quality model

In many coastal ecosystems phytoplankton and suspension feeding bivalve production are tightly coupled (Dame, 1996). That suspension-feeding bivalves can play an important role in controlling phytoplankton abundance in coastal systems is well established (e.g., Officer *et al.*, 1982; Cloern, 1982; Nichols, 1985; Alpine and Cloern, 1992). Through their feeding activity bivalves can alter nutrient dynamics (Dame *et al.*, 1984; Dame and Libes, 1993), affect sediment composition and nitrogen cycling (Kaspar *et al.*, 1985), strongly affect carbon budgets (Rodhouse and Roden, 1987) and alter the composition of both benthic and planktonic

2003年６月12日受理（Received on June 12, 2003）
* Virginia Institute of Marine Science, College of William and Mary. P. O. Box 350, Wachapreague, VA, USA, 23480
Email: luck@vims.edu Contribution number 2584 from the Virginia Institute of Marine Science

assemblages (Tenore *et al.*, 1982). Wild populations of bivalves have been implicated in some instances (Officer *et al.*, 1982, Cloern, 1982; Newell, 1988; Dame, 1996), but much evidence has come from bivalve aquaculture operations, including mussel culture in the Rias of northwest Spain (Tenore *et al.*, 1982) and New Zealand (Kaspar *et al.*, 1985) and oyster culture in Killary Harbor, Ireland (Rodhouse and Roden, 1987) and the Marennes-Oléron Bay in France (Bacher, 1989), that suspension-feeding bivalves affect phytoplankton dynamics on large scales.

The reciprocal is, of course, also true; phytoplankton production strongly affects bivalve production. In a review of trophic dynamics in temperate estuaries Heip *et al.* (1995) concluded that the ecological carrying capacity of bivalve populations in estuaries and coastal bays is often constrained by phytoplankton production. For bivalve aquaculture, which seeks to maximize shellfish production, phytoplankton production may determine the *exploitation carrying capacity*-the maximum yield of market-size individuals within a particular environment. Carver and Mallet (1990) showed that the exploitation carrying capacity of a coastal embayment in Nova Scotia for mussel production varied with temporal variations in food supply. A series of models developed to predict the exploitation carrying capacity for oyster aquaculture production in Marennes-Oléron Bay in France (Bacher, 1989; Héral, 1993; Bacher *et al.*, 1997) have been used to predict optimum stock size for maximizing production in this estuary. Similarly, Ferreira *et al.* (1998) developed a carrying capacity model for oyster cultivation in Carlingford Lough, Scotland. In the Oosterschelde estuary in the Netherlands, Smaal *et al.* (2001) found that mussel production was limited by phytoplankton production. They estimated the carrying capacity of the estuary for mussel culture before and after large-scale hydrographic modifications and discussed how adapting aquaculture practices in the context of food limitations helped the industry to maximize production.

Hard clam (*Mercenaria mercenaria*) aquaculture has expanded dramatically over the past decade along much of the United States' Atlantic coast and the northeast Gulf of Mexico. In Virginia, growth in this industry has been especially dramatic. A conservative estimate places the total standing stock of cultured clams in Virginia in excess of 500 million clams (estimate based upon federal crop insurance program statistics). Most of this aquaculture occurs in small tidal creeks that empty into the Chesapeake Bay and shallow embayments behind coastal barrier islands (Fig. 1). In many of these areas clam aquaculture has grown to a scale at which it may be reasonable to ask if clam production is close to the limit set by phytoplankton production, i.e., it may be at, near or even exceeding exploitation carrying capacity.

Additionally, the clam aquaculture industry faces threats from changing land use practices in the adjacent watersheds. Rapidly changing demographic trends in the region are leading to the replacement of farmland by residential and industrial uses. The potential impacts of this changing use on water quality and clam aquaculture are presently unknown.

In this paper we will (1) give a brief descrip-

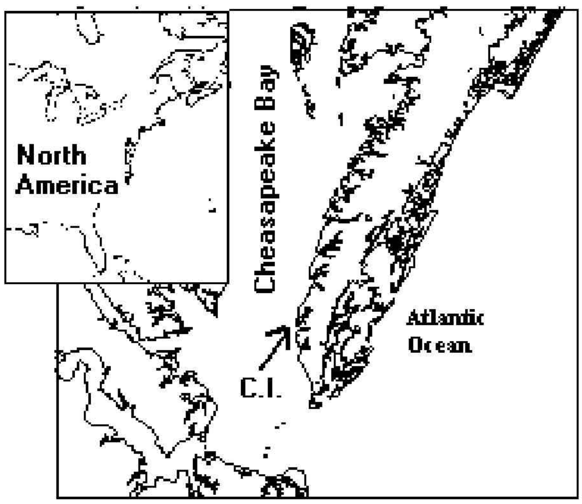

Fig. 1. Region of study. Clam aquaculture is taking place in the tributaries of the lower Chesapeake Bay and the coastal embayments on the Atlantic coast. C.I. denotes the location of Cherrystone Inlet.

tion of the farming practices for hard clams, (2) detail some preliminary calculations that reveal the potential strength of the interactions between phytoplankton production and clam production in one tributary, and (3) describe a modeling effort that is currently underway to link watershed loading, water quality and clam production models to provide a tool for predicting effects of changing land-use practices on water quality, phytoplankton production and clam aquaculture production.

Hard clam aquaculture

Detailed descriptions of the production practices for farming hard clams are given in Manzi and Castagna (1989) and Castagna (2001). Briefly, larvae are spawned and reared in hatcheries in seawater ranging from 20 to 35 ppt. Post-set juveniles are generally raised in land-based, flow-through nursery systems until they reach a size of 3-4mm in shell height. At that time they are placed into one of several types of field nursery systems-either fine mesh bags, sand-filled trays or floating upweller systems-and grown to a size range of 10-15mm. Clams are then planted in the low intertidal and subtidal zone in sand and sand-mud mix bottoms for grow-out to market size. Generally clams are planted in rows approximately 4 m $\times$ 18m at densities ranging from 550 to 1650 clams m^{-2} and covered by polyethylene netting that serves as predator protection. A typical clam farm may include several hundred such nets (Fig. 2). The grow-out period ranges from 18 to 30 months with most clams harvested within 24 months of planting.

Fig. 2. Aerial view of a clam farm in Chesapeake Bay, U.S.A. Each rectangular net is approx. 4 m $\times$ 18m and covers 50,000 clams.

In some of the most densely planted tributaries and embayments clam farmers have begun to observe declining growth rates over the past few years. One possible explanation for this observation is that these areas may have exceeded the exploitation carrying capacity and clam production may be limited by primary production in these systems.

Estimating links between clam production and water quality

To provide a first approximation of the effects of clam culture on water quality and of the possibility that clam production may be limited by phytoplankton production in these systems, we performed some simple calculations for Cherrystone Inlet, a small tidal embayment on the eastern margin of the Chesapeake Bay (see Fig. 1). From the aquaculture industry we have an estimated standing stock of cultured clams in system of 45×10^6. The volume of Cherrystone Inlet at high tide is $15.4 \times 10^6 m^3$ and the volume of the time averaged tidal prism is $5.8 \times 10^6 m^3$ (Kuo *et al.*, 1998). We used the filtration rate formula reported by Hibbert (1977a, b) for *Mercenaria mercenaria* at 25 ℃ (typical summertime water temperatures in this area): $FR = 0.063 \cdot L^{0.834}$, where L = shell length and FR has units of L $indiv^{-1}$ hr^{-1}. Multiplying by the total number of clams and dividing into the total and tidal prism volume estimates then provides rough estimates of the clams' filtration volume relative to volumes in the embayment (Table 1). Data from the clam industry indicates that annual harvest of clams from this region is approximately 20×10^6 with an average shell height of 60mm. Using a shell height to dry weight regression from Walne (1972) and value for tissue nitrogen = $0.1 \cdot$ dry tissue weight (Hawkins *et al.*, 1985), we estimate the amount of nitrogen removed annually from the system

Table 1. Preliminary estimates of some of the effects of clam aquaculture on filtration on water quality in Cherrystone Inlet. (N_H=nitrogen removal from harvest, N_{ATM}=nitrogen loss as N^2 to the atmosphere, N_{EX}=nitrogen excretion rate, primarily as ammonia.

Summertime filtration rate			Nitrogen uptake and release		
Total filtration rate	% of tidal exchange	Time to filter creek volume	N_H	N_{ATM}	N_{EX}
1.6 x 10^6 m^3 day^{-1}	28%	10 days	18,000kg yr^{-1}	36,000kg yr^{-1}	900kg day^{-1}

by harvesting (Table 1). Estimates by Hibbert (1977a) on the production of feces and pseudofeces by clams and measurements reported by Newell *et al.* (2002) for rates of nitrification and denitrification of bivalve biodeposits were used to compute the amount of nitrogen lost as N_2 to the atmosphere as a result of clam feeding (Table 1). Finally, we estimate nitrogen excretion rates from these clams based on Hibbert (1977a) (Table 1).

We emphasize that all of the estimates in Table 1 are preliminary. Our intention in making these estimates was to provide an initial evaluation of whether clam aquaculture in this system might have reached a scale at which it was reasonable to hypothesize that it is near carrying capacity or that clams are having a significant effect on water quality and phytoplankton dynamics. Evaluating these hypotheses will require an integrated modeling approach. As noted above, land use in the watersheds around these small tidal creeks and embayments in which clam aquaculture is occurring is changing. It is likely that this change will affect the inputs of nutrients, sediments and freshwater into these water bodies, which in turn will have consequences for clam production. In the sections below we provide an overview of a modeling approach that we are taking to address these issues.

Model Description

Carrying capacity models

Smaal *et al.* (1998) reviewed the requirements for modeling bivalve carrying capacity in coastal ecosystems. In doing so, they outline a number of sub-models that are required to provide input to an ecosystem-level model that estimates maximum production level. In short, hydrodynamic and sediment sub-models are used to characterize movement of materials in the water column and between the sediment/ water interface, respectively. A physiological sub-model incorporates bivalve feeding and energetics on a size-specific basis, while the population sub-model builds in the planting densities and harvest schedules along with cohort growth and mortality. Output from these sub-models together with data on physical characteristics (e.g., light, T & S) and nutrient loadings are then used in a system-level model to estimate production capacity. Below we discuss in greater detail how each of these sub-models are being developed for Cherrystone Inlet.

Hydrodynamic submodel

A hydrodynamic sub-model called HEM3D (Hydrodynamic Eutrophication Model 3D) has been developed at Virginia Institute of Marine Science as part of a dynamic water quality model. This model has been calibrated and verified in a number of systems such as York River, James River, Mobile Bay and Florida Bay with relative success and has been selected by the U.S. Environmental Protection Agency as one of the standard model codes for water quality application. The hydrodynamic sub-model requires a number of inputs. The geometry (shape) and bathymetry of the system is required to construct grid configuration and bathymetry (each model cell is given an average depth value). Boundary condition specifications of forcing function(s) (e.g., tidal elevation, velocity, and usually salinity) at the open boundary, as well as discharge at the upstream boundary must be specified. Runs of the model are facilitated by proper initialization values of model parameters, especially those that require a relatively long time to equilibrate, such as salinity. Finally, specification of other inputs facilitates the calibration process (e.g., bottom friction adjustment). For Cherrystone Inlet the geometry and bathymetry data have already been collected and the grid system developed (Kuo *et al.*, 1998). Boundary conditions for all forcing functions have been established and proper initialization and calibration terms incorporated. Simulation with a precursor to this model, a tidal prism water quality model conducted by Kuo *et al.* (1998), was the first attempt using coupled hydrodynamic, water quality and watershed models in the Cherrystone Inlet. The simulated results of salinity, chlorophyll, DO, total carbon, total nitrogen and phosphorus provided reasonable predictions compared to bi-monthly measured field data (Kuo *et al.*, 1998).

The state variables in this sub-model include tidal elevation at each cell in the horizontal and velocity, salinity, and temperature at cells in both the horizontal and the vertical directions. Process variables are derived from these, and include transport processes such as density driven circulation. Advection and dispersion influence the flow field as well. Pending further field verification and calibration this sub-model will be used to generate the transport features that drive the system-level model.

Resuspension/sedimentation model

This sub-model, which is currently under development, will simulate fluxes of sediments and other materials between the water column and the bottom. In order to calculate suspended sediment concentration in each cell of the model domain, flow velocities are provided to the sediment sub-model by the hydrodynamic sub-model. In addition, sediment settling velocities are specified for each size class of the sediment. Time series of sediment concentrations at the upstream and open boundaries are used as the input sediment fluxes to the model domain. The erosion and deposition rate, representing vertical sediment fluxes between the water column and the bottom, are calculated based on bottom shear stress and the critical shear stress.

The sediment sub-model is being calibrated to simulate the temporal and spatial variations of sediment concentrations within the model domain. The calculated sediment concentration in each cell will be used to determine food quality and light attenuation factors for the growth of algae. A benthic compartment in general and a clam sub-model in particular are important to include as both sink and source for nutrients and particulate matter.

Clam feeding and physiological sub-model

The purpose of the clam feeding and physiology sub-model is to link the feeding, growth, energetics and excretion by clams to the general system-level model. Data are available on the feeding, growth and energetics of wild *M. mercernaria* throughout its range (reviewed by Grizzle *et al.*, 2001). Feeding rates vary with body size, temperature and salinity; univariate relationships with each of these factors have been reported in a large number of studies (e.g., Loosanoff, 1939; Coughlan and Ansell, 1964; Hamwi, 1969; Hibbert, 1977a,b; Doering and Oviatt, 1986; Bricelj, 1984; Walne, 1972). Feeding rate also varies as a function of food concentration (Tenore and Dunstan, 1973), silt content of the seston (Hamwi, 1969; Bricelj and Malouf, 1984) and phytoplankton species composition (Rice and Smith, 1958; Walne, 1972). Relationships between respiration rate and body size (Loveland and Chu, 1969) and respiration rate and temperature (Hamwi, 1969; Hibbert, 1977b) have been published for *M. mercenaria*. Hibbert (1977a,b) developed an energy budget for this species growing on mudflats in Southampton, England.

In their extensive review of hard clam physiological ecology, Grizzle *et al.* (2001) report growth rates for *M. mercernaria* that range from 0.42-1.11mm (growth in shell height) week^{-1} and average time to market size (25.4mm shell thickness) ranging from 2.1 to 13.0 years over a geographical range from Prince Edward Island, Canada, to the Florida Gulf coast. While they observed a latitudinal gradient in growth rate, they noted that over a broad geographical range (New York to Florida) variation in growth rate within an estuary generally exceed that attributable to latitude (Grizzle *et al.*, 2001).

The basic construct of the physiology submodel is straightforward:

Growth = Consumption − Respiration − Egestion − Excretion.

Consumption is a function of filtration rate and food availability. Filtration rate and respiration vary as a function of temperature and body size as discussed above. Because the cultured clams have been artificially selected for high growth for several decades by aquaculture industry, it will be necessary to measure each of these variables for cultured animals in Cherrystone Inlet, rather than relying on published values for wild clams. When completed this sub-model will output growth rates from one size class to another that will serve as input to the population sub-model.

Clam population sub-model

For wild populations of bivalves obtaining reliable parameter estimates for the population sub-model can often be the most difficult part of developing carrying capacity model. Uncertainties surrounding estimates of reproductive output, larval survival and post-settlement mortality for field populations all make it difficult to obtain accurate predictions. For aquaculture, however, the parameter estimates are straightforward. The number and size of clams planted by the industry, the growth rate between size classes and the harvest of clams by the industry, along with a small, but defined, non-harvest related mortality are the primary parameters of interest. These values will serve as inputs to the population sub-model and the measured growth rates will be compared to predicted values based upon the linked sub-models.

System-level model

Output from the various sub-models serves as input for the system-level model that computes several state variables including primary production and bivalve biomass for each cell at the specified time step. The role of the hydrodynamic sub-model is to provide the transport quantities induced by the physical process, such as fluxes between the boxes and the mixing within a box. The sediment resuspension/deposition model calculates the suspended matter concentration, which in turn determines the food quality and light attenuation. The physiology sub-model drives the growth of clams that feeds into the clam population sub-model to drive cohort production estimates.

Because primary production varies temporally and spatially within estuaries, a dynamic modeling approach as described above is required to estimate the exploitation carrying capacity for bivalve aquaculture (Smaal *et al.*, 1998). Thus, once developed and calibrated, we will run the model under varying conditions of nutrient loading to investigate how clam production is affected by nutrient loading (either from the Bay or the watershed) and under varying clam stocking densities to evaluate aquaculture practices. Additionally, we will explore the effects of clam aquaculture on water quality within the system by running the model under various stocking densities (including no clam aquaculture) and estimating chlorophyll and light attenuation levels.

Watershed loading sub-model

A unique feature of our project to estimate carrying capacity for clam aquaculture is an ongoing effort to link a watershed loading model to the system-level water quality model. In order to understand and quantify the effects of changing basin scale land-use patterns, a watershed model is needed. The HSPC (Hydrologic Simulation Program C language) will be used for simulating the watershed hydrology and associated water quality parameters on pervious and impervious land surface and in streams and well-mixed impoundments. Sediment and nutrient loadings in the model are transported from land to the receiving coastal waters. Inputs to this model include precipitation, soil type and land-use within the watershed. Outputs will include freshwater discharge, nutrient inputs and sediment loading, each of which serves as input into the system-level water quality model. Data collected from within the watershed over the next two years will serve as initial input to the model for the purpose of modeling current conditions. Subsequently, we will vary these inputs to explore how changing conditions within the watershed affect water quality and clam production.

Discussion

Clam aquaculture is an important and growing industry in the U.S. that is replacing fisheries on over exploited wild stocks and providing economic development in traditional coastal fishing communities. Sustaining clam aquaculture in the coastal waters of the U.S. will require an improved understanding of the inter-relationships between coastal land-use, water quality, primary production and clam production. The modeling approach described here will provide an important suite of tools for advancing our understanding of these linkages.

Initial estimates from a single embayment in Virginia suggest that clam aquaculture has developed to a scale at which system-level impacts on water quality and phytoplankton dynamics may be evident (Table 1). Understanding those effects, as well as how watershed inputs and water column dynamics affect the growth and production of hard clams, requires an integrated modeling effort such as the one we described here. This effort is still underway with each of the sub-models currently being refined and field data being collected for parameterization, calibration and verification.

When complete, the model output under current conditions will provide an understanding of the relationship between current clam production levels and primary production within Cherrystone Inlet. If the model results indicate that the Cherrystone Inlet system is near or has exceeded exploitation carrying capacity, then the explanation for the reported decrease in clam growth will lie in the relationship between basin-wide phytoplankton production and clam stocking densities. Conversely, if clam production is not limited by primary production, then either local growing conditions (e.g., sediment changes) or genetic condition of the stocks may be the cause of this pattern.

The utility of this model goes beyond explaining the current growing situation in Cherrystone Inlet. It will permit us to explore various scenarios of stocking density, seasonal and inter-annual variations in primary production, and altered nutrient loading to assess their impacts on clam production in the system. Furthermore, although the specific formulation of the model in this instance will be for Cherrystone Inlet, once developed and calibrated, it should provide a useful starting point for describing the relationship between primary production and clam aquaculture in other tidal creeks and embayments throughout the mid-Atlantic region of the U.S. With the addition of basin-specific inputs on geometry, bathymetry, tidal elevations and currents, salinity and boundary conditions the model will serve to estimate exploitation carrying capacity for clam aquaculture in any system in the mid-Atlantic region.

By coupling the clam physiology and population sub-models with the hydrodynamic-based water quality model and linking it to a watershed loading model, we will achieve a powerful tool for use by natural resource managers and local governments that goes beyond traditional carrying capacity models. For instance, this region is experiencing growing development pressure, especially along the waterfronts adjacent to clam growing areas. The coupled models described here will permit us to run various hypothetical watershed development scenarios and predict the impacts on water quality and clam production. This information will be valuable in permitting informed decisions about coastal development and its impacts on a valuable aquaculture industry.

Acknowledgments

Development of various components of the water quality and watershed loading models have been funded by the Maryland Department of the Environment and Virginia Department of the Environmental Quality. Development of the carrying capacity model is being supported by NOAA Sea Grant Program.

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水研センター研報，別冊第１号，133－142，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 133-142, 2004

Environmental change in the coastal environment: challenges for the selection and propagation of filter feeding species in aquaculture, stock enhancement and environmental rehabilitation.

Roger MANN*

Abstract Selection of species for aquaculture, fishery stock enhancement and environmental rehabilitation or restoration in the coastal zone requires consideration of the fact that species have evolved over geological time whereas changes in the coastal environment have occurred predominantly over recent historical time, often with the largest changes occurring within the past decades of human activity. The evolutionary issue is particularly noted with filter feeding molluscs, where extant species supporting both major natural fisheries and aquaculture have ancient lineages and evolved in environments that may have differed considerably from the locally turbid, nutrient enriched, disturbed (through watershed change and local activity) waters in which they now survive. We cannot presume that native species are strongly selected to survive in the environments in which they currently reside. Neither can we presume that they will be successful candidates for aquaculture, fishery stock enhancement, environmental rehabilitation (the restoration of ecological services in community structure), or environmental restoration (restoration of native community structure with associated ecological services). Watershed and coastal use impacts have, over recent human history, altered community structure in coastal waters, and diminished the ability of surviving community members to perform the ecological services that are one end product of their evolution. A challenge is therefore presented to students of intensive species culture, extensive fishery enhancement, and ecological rehabilitation or restoration: how to best use the tools of husbandry in concert with large and small scale environmental manipulation to promote progress in the designated area of interest? Ecological rehabilitation or restoration centered on cornerstone filter feeding species must employ local environmental rehabilitation, but this will only be successful if it is accompanied by a wider commitment to watershed management protocols that protect all life history stages, including the delicate early stages. A numerical argument for this approach, based on Paulik life history models, will be presented. Intensive aquaculture, by comparison, may be able to progress in marginal environments where delicate early life history stages are cultured in controlled situations, thus limiting mortality, before transfer to open systems. Fishery enhancement resides between these options, where a dual role of supplementing local reproduction is balanced against increased exploitation of commercial product.

Key words: bivalve molluscs, aquaculture, stock enhancement, environmental rehabilitation

2003年６月12日受理（Received on June 12, 2003）
*[1] Virginia Institute of Marine Science, Gloucester Point, VA 23062 USA, phone: 804/684-7360, FAX: 804/684-7045,
Email: rmann@vims.edu

Selection of species for aquaculture, fishery stock enhancement and environmental rehabilitation or restoration in the coastal zone requires consideration of the fact that species have evolved over geological time whereas changes in the coastal environment have occurred predominantly over recent historical time, often with the largest changes occurring within the past decades of human activity. A history of the traits driving selection of aquaculture species is given by Mann (1984a), and emphasizes the roles of species low in the food chain with physiologically tolerant adult stages and reproductive cycles that are easily subject to control and manipulation. Filter feeding bivalve molluscs fit these requirements.

The evolutionary time frame is particularly important with filter feeding molluscs, where extant species supporting both major natural fisheries and aquaculture have ancient lineages and evolved in environments that may have differed considerably from the locally turbid, nutrient enriched, disturbed (through watershed change and local activity) waters in which they now survive. This grouping includes extant oysters, mussels and clam species of commercial interest, especially those under active aquaculture. Oysters are a notably primitive form, exemplified by the loss of the second adductor to the monomyarian form. They have survived by migrating over geological time into geologically ephemeral estuarine environments, being served in this purpose by their complex life history employing pelagic larval forms. Recent commentaries on the evolution of the larval form summarized in McEdward (1995) and Hall and Wake (1999) emphasize why the combination of these "dual" evolutionary paths of the adult and larval forms in response to stage specific selection pressures has left the "primitive" bivalves so well equipped to respond to rapid (in the evolutionary time frame) changes in local environments that would typically lead to local extinction. Oyster populations in the Chesapeake Bay provide an excellent example of successful invasion in geologically ephemeral environments. The current bay is a product of 8,000 years of Holocene sea level rise (Haven and Whitcomb, 1983; Hargis 1999). Recent fossil deposits including oyster shells can be found many kilometers offshore of the current shoreline in depths corresponding to earlier sea level stanzas. These illustrate both the changing environment with sea level rise over recent geological time and the accompanying "migration" of the oyster populations.

Substantial changes have occurred in both the watershed biology and physical environment of the Chesapeake Bay since the arrival of European colonists. The past four centuries have been marked by deforestation for agriculture, urban development on the shoreline of the bay or its tributaries, and increasing, and mostly uncontrolled exploitation of the fishery resources for much of the period. The human population in the Chesapeake Bay watershed has reached approximately 15 million with projected growth rates at double the national average for the next twenty years. The record of watershed change is abundant from both direct public records and indirect evidence from sources such as pollen records of land use change. Extensive records of recent and current land use patterns are available from the U.S. Environmental Protection Agency world wide web site at http://www. chesapeakebay. net/bayprogram with summary information changes in land use practices such as no till farming and shoreline buffer zones designed to reduce non point source sediment and nutrient runoff into the bay. In addition, changes in the bay and tributary morphologies have resulted from river dredging and shoreline development. Fundamental changes in the biology of the aquatic system have been caused by over fishing and critical environmental degradation. Failure to return shell to the bottom after oyster harvest has resulted in once significant three dimensional reef structure being reduced to patchy monolayers of substrate for extant oyster populations with accompanying change in both the complexity of the associated communities and the ecological services they provide (compare data from Baylor 1894 with

Luckenbach, Mann and Wesson 1999). This rapid, in an evolutionary time frame, change in local environment has clear implications for changes in community structure in the local community (Mann, 2001). We cannot presume that target species are strongly selected to survive in the environments in which they currently reside, as opposed to those in which they evolved. Neither can we presume that they will be successful candidates for aquaculture, fishery stock enhancement, environmental rehabilitation (the restoration of ecological services in community structure), or environmental restoration (restoration of native community structure with associated ecological services) in these rapidly changing environments. Indeed, this realization has been a driving force for the use of non-native species in both intensive aquaculture and fishery enhancement in recent decades (see Mann 1979, 1984b; Rosenfield and Mann, 1992).

A challenge is presented to students of intensive species culture, extensive fishery enhancement, and ecological rehabilitation or restoration: how to best use the tools of husbandry in concert with large and small scale environmental manipulation to promote progress in the designated area of interest? Ecological rehabilitation or restoration centered on cornerstone filter feeding species must employ local environmental rehabilitation, but this will only be successful if it is accompanied by a wider commitment to watershed management protocols that protect all life history stages, including the delicate early stages. The cost-benefits of each component of this restoration or rehabilitation strategy on the performance of a target species in culture or as a fishery are of direct interest. Can specific management actions be targeted at improving the response of the most susceptible life history stages to specific stresses? A numerical approach to this question is offered by consideration of Paulik life history models.

Paulik (1973) provide a useful conceptual tool for quantitative descriptions of the stock - recruit relationship in commercially exploited marine fish species. Examples are discussed in Rothschild (1986). The graphical presentation of the concept, the Paulik diagram, consists of a four-quadrant plot. Quadrant 1, proceeding in a clockwise direction, is the desired stock-recruitment function. It is constructed from the end product of the other three quadrants. The second quadrant relates stock size to egg production and thus incorporates absolute number of fertile individuals, demographics through size specific fecundity, and the integrated effect of environment and prior feeding history on fecundity. The third quadrant relates egg production to larval production and incorporates spawning synchrony, spawning behavior where applicable, and density dependent fertilization success. The fourth quadrant describes the relationship of larval production to recruitment to the subsequent spawning generation. In fish populations this does not involve a transition from pelagic to attached or sessile benthic form, although a larval to adult metamorphosis is included. Application of Paulik models to benthic invertebrates with complex life histories dictates that this quadrant include larval growth and loss to predation and dispersal to hostile environments, settlement and metamorphosis, and post settlement growth to sexual maturity. Applications of such models to cultured benthic species are somewhat rare, yet the structure provides a useful tool for integrating studies of individual life history stages. Of particular importance in the context of stresses acting on coastal ecosystems is the ability to quantitatively describe the impact of a stress on one life history stage on a separate but subsequent life history stage. In the coastal zone where increasing eutrophication and other pressures can disproportionately impact one life history stage over another in isolated test challenges the utility of this holistic approach is both obvious and powerful.

Mann and Evans (1998) adapted the approach of Paulik (1973) in estimation of oyster, *Crassostrea virginica*, standing stock, larval production and advective loss in relation to observed recruitment in the James River, Virginia. The present illustration simplifies this approach using a virtual population to examine the effects of three parameters on recruitment in subsequent generations. These parameters are (1) varying egg production by varying age specific mortality of the parent population as a proxy for disease impacts, (2) varying duration of larval period in response to sub-optimal feeding conditions, and (3) varying loss to advection related to estuarine tidal exchange. All three variants are examples of the effect of possible environmental stresses on critical life history stages. Others can be examined by the method described below as appropriate to the location under stress.

Methods

To summarize and simplify Mann and Evans (1998), recruitment to the 25mm size class is estimated from larval supply thus:

$(F_{tot} \times F_q \times F_s \times F_d \times F_f) \times (1 - exch)^{2d} \times (1 - L_{mort})^d \times P_{sub} \times P_{foul} \times P_{met} \times (1 - J_{mort})^{dp}$

where:

F_{tot} is total egg production and estimated from size specific fecundity. It is a cumulative total for all individuals (F_{ind}) in all size classes and typically estimated from length:dry weight estimators. In the current illustration all sizes below 40mm are considered young of the year (spat) and do not contribute to spawning, and fecundity is estimated from relationships given in Thompson et al (1996), and Mann and Evans (1998).

F_q is a sex ratio modifier. Cox and Mann (1992) suggest parity in sex ratio. Given the lack of other data a single sex ratio modifier, F_q, with a value of 0.5 (50% female in all size classes) is used in this illustration.

F_s: F_{ind} and hence F_{tot} can be modified based on salinity (S) effects. Mann and Evans (1998) suggested the following estimators for F_s:

if $S > 13.5$, $F_s = 1.0$

if $S < 13.5$, then

$F_s = [(S - 8.0)/(13.5 - 8.0)] \times 1.0 = (S - 8.0)/5.5$

F_d modifies fecundity for disease effects with values ranging from 1.0 to 0.0. In the present illustration it varies from 1.0 to 0.75 (a 25% reduction based on disease impact).

F_f describes a density dependent multiplier for fertilization efficiency with values from 1.0 (100% fertilization) to 0.0 (no fertilization). It is based on Levitan (1991) where:

log % fertilization $= [0.72 (\log OD) + 0.49]$ or,

% fertilization $= [0.49 \times OD^{0.72}]$

where OD is oyster density in numbers m^{-2}. In the present illustration it is rewritten thus:

$F_f = 0.0049 \times OD^{0.72}$

Production of larvae (strictly speaking embryos or fertilized eggs) m^{-2} is therefore estimated by ($F_{tot} \times F_q \times F_s \times F_d \times F_f$) in units of larvae m^{-2}.

$(1 - exch)^{2d}$: Mann and Evans (1998) estimated retention of the larvae within the James River during planktonic development using the three dimensional flow model of Hamrick (1992a, 1992b) to provide source and sink data at scales within the estuary. For the present illustration a simple dilution function is used that assumes uniform dispersal within the estuary and proportional loss on each tidal cycle, that is larvae are assumed to be neutrally buoyant and exert passive swimming behavior in response to oriented stimuli. Thus larval numbers decreased with days with the duration of planktonic development by the function $(1 - exch)^{2d}$ where *exch* is proportional volumes *exch*anged on each tide. The value of *exch* varies in the current study between 0.1 and 0.2 (0.2 equals a 20% *exch*ange per tidal cycle) and d is the duration of the larval development (=planktonic) period. The correction $2d$ is used with a simple assumption of 2 tidal *exch*anges per day. In the current study d varies from an optimum of 21 days, based on values from Mann *et al* (1994), Mann and Evans (1998), Bochenek *et al* (2001) and Powell *et al.* (2002), to a sub-optimal value of 25 days based on assumed reduction of feeding and hence growth in low salinity and/or high turbidity regions.

$(1 - L_{mort})^d$ estimates larval mortality in the water column. L_{mort} is the daily larval mortality rate (a proportional value between 1.0 (all died) and 0.0 (no mortality)). Survival is $(1-L_{mort})$ for a period of one day or $(1-L_{mort})^d$ for a d day planktonic development period. For the current illustration L_{mort} is set at 0.05, 0.06, 0.07. 0.1 and an extreme value of 0.25. The decreasing exponential relationship insures a gradual decreasingly sensitive response to increasing values of *d*. Modification of the original number of larvae to account for dispersal loss and mortality provides an estimate of larvae surviving to immediate pre-metamorphic size. The transition to an attached benthic form requires successful location of substrate, that the substrate not be occluded by competing organisms, and that the larvae have sufficient energy reserves to complete the metamorphosis to a juvenile feeding form.

P_{sub}, a dimensionless modifier with a value between 1.0 and 0.0, describes the probability of finding suitable substrate. The time scale and availability of shell substrate is critical to successful recruitment (Morales-Alamo and Mann 1990). Consider that a shell layer one-cm thick covering one-sq. m of bottom has a volume of 10L. For the current illustration a premise is adopted that a shell layer a minimum of one cm thick is required to offer a suitable substrate. P_{sub} is estimated thus:

if shell volume $>$ 10L m^{-2}, $P_{sub}=1.0$

if shell volume $<$ 10L m^{-2},

$P_{sub}=0.1\times$ Shell Vol. (in liters)

P_{foul} describes proportional occupation of the substrate by competing organisms and varies between 1.0 (no fouling) to 0.0 (complete preclusion of settlement). Rheinhardt and Mann (1990) suggest a value of $P_{foul}=0.33$ based on field studies in the James River. For the current illustration a constant value of 1.0 is employed.

P_{met} describes the probability of successful completion of metamorphosis to the attached form on a 1.0 (all survive) to 0.0 (no survival) scale. For the present application the value is set at 0.20.

Recruitment to the benthos is therefore estimated from larval supply values by incorporating $(1-exch)^{2d}$, $(1-L_{mort})^d$, P_{sub}, P_{foul} and P_{met} thus:

$$[(F_{tot}\times F_q\times F_s\times F_d\times F_f)\times(1-exch)^{2d}\times(1-L_{mort})^d \times P_{sub}\times P_{foul}\times P_{met}]$$

$(1-J_{mort})^{dp}$ modifies this estimator for post settlement mortality and growth rates, both of which are known to be size dependent (Roegner and Mann, 1995). Mann and Evans (1998) describe daily juvenile mortality rate as J_{mort} (proportional with a value between 0.0 and 1.0). Survival is $(1-J_{mort})^{dp}$ where dp is the number of days to grow to a defined size. Based on values of J_{mort} in Roegner and Mann (1995), Mann and Evans (1998) suggest a cumulative mortality to 8 mm length of 93 % over a 28 days period, a calculated value for J_{mort} of 0.09. Thus $(1-J_{mort})^{dp}$ for the current study is set at 0.07 to 8 mm length. Above this length J_{mort} is lower and set at 0.05 for another 25 days until a size of 25 mm when the surviving individuals are considered recruits to the subsequent generation (Eggleston, 1990). For the current illustration $(1-J_{mort})^{dp}$ incorporates two mortality rates with a cumulative mortality value for the pre-metamorphosis larvae to 25 mm size class, including a P_{met} value of 0.20 is 99.84 %, or a proportional survival of 0.0016.

Demographics for a virtual population were generated from a data set describing Horse Head Reef in the upper James River for the period 1993-1996 (National Oceanic Atmospheric Administration Chesapeake Bay Stock Assessment funds to R. Mann and J. Wesson, Virginia Marine Resources Commission). This population was chosen because it was (a) stable over that period with respect to recruitment, total oyster density, and oyster demographics, and (b) suffered essentially no mortality due to disease. The size frequency distribution (in 5m size classes) was converted to an age frequency demographic using an a modified Von Bertalanffy plot with growth oscillation corresponding to seasonal change in growth rate (Evans and Mann, unpublished data based on field data from Horse Head reef in the James River, Virginia, by Mann and Morales). This takes the form:

$L_t=L_{inf}\ (1-\mathrm{e}^{\text{芋}K\text{摭芋}t_0\text{輆}A\text{芋}B\text{箏}})$,

where: $A=C\sin(2\pi(t-t_s))\ /\ (2\pi)$, and $B = C \sin(2\pi(t_o - t_s)) / (2\pi)$

The parameters are: L_t is the length at time t, L_{inf} is asymptotic length set at 120mm based on field observations, K is the growth constant, t_o is age at which length is zero, C is the amplitude of the growth oscillation, and t_s is the starting point of the oscillation with respect to $t=0$. The parameters of this function were estimated by fitting a rearranged function to the size increment data of a data set which records serial increase in length over time so that we have values of L_1, L_2, and so on. The rearranged function is:

$$L_2 = L_{inf}(1-(1-L_1/L_{inf})\, e^{-K((t_2-t_1)+A'-B')})$$

where $A' = C\sin(2\pi(t_1 - t_s))/(2\pi)$,

and $B' = C\sin(2\pi(t_2 - t_s))/(2\pi)$

The parameters were estimated as follows: $K=0.204$, $t_o=0.36$, and $t_s=0.608$.

The virtual population demography is illustrated as population A in Fig. 1A, together with a series of subsequent populations (B-E inclusive) generated by gradually increasing age specific mortality (illustrated as cumulative mortality in Fig. 1B) specifically chosen to simulate the effects of increasing disease prevalence and intensity. It is notable that the extreme population, E, represents an approximation of current disease tolerance in the most selected strains under typical disease challenge in medium salinity waters. Each population has the 25mm size class, here considered the young of the year recruits or zero class, set at 100 oyster m^{-2}. This corresponds to the end point of the above "recruitment to the benthos" estimator. In all simulations, which are run as a sequential spreadsheet in Microsoft Excel the barometer for successful recruitment of a subsequent generation is attaining a 25mm size density of 100 oyster m^{-2}.

Results

The simulation was run for a single generation time frame with each of A-E as the starting demographic under various scenarios and the end points illustrated in Fig. 1C – 1H. Although these are just a subset of the many options that can be run with the simulations they illustrate the following important points: Under low tidal exchange and optimum larval development the recruitment values are very high even with high larval mortality rates (Fig. 1C). Consider, however, that the employed scenario uses many optimal conditions including no reduction in fecundity attributable to salinity, no shell limitation and only modest competition for substrate. This is very much an optimal scenario.

(a) Increasing larval duration by only 4 days reduces recruitment considerably (Fig. 1D).

(b) Increasing tidal loss to 20% drives all recruitment values below the critical $100 m^{-2}$ even with everything else at optimum (Fig. 1E).

(c) Reducing fecundity by 25% as a proxy for impact of disease and/or salinity has a proportional effect (Fig. 1F).

(d) Reducing fecundity by 25% and increasing tidal loss to 15% provides options for all population structure from A through E to recruit at $< 100 m^{-2}$ depending on larval mortality rate, even with all other factors optimized (Fig. 1G, 1H).

These examples underscore the very non-linear response of recruitment to various combinations of tidal *exch*ange, reduced fecundity, and larval duration as we move away from an optimal combination. They also illustrate that even the most stable population structure, A in Fig. 1A, to produce marginal recruitment even with shell and competition (P_{sub} and P_{foul}) optimized and with no consideration of greater impact of post settlement mortality. In a large number of slightly less than optimal scenarios even a population with increased mortality under sustained disease pressure – E in Fig. 1A – are prone to inadequate recruitment.

Discussion

These limited simulations underscore the need for holistic approaches to restoration or response to multiple stresses. Approaches that focus on only optimizing one life history stage

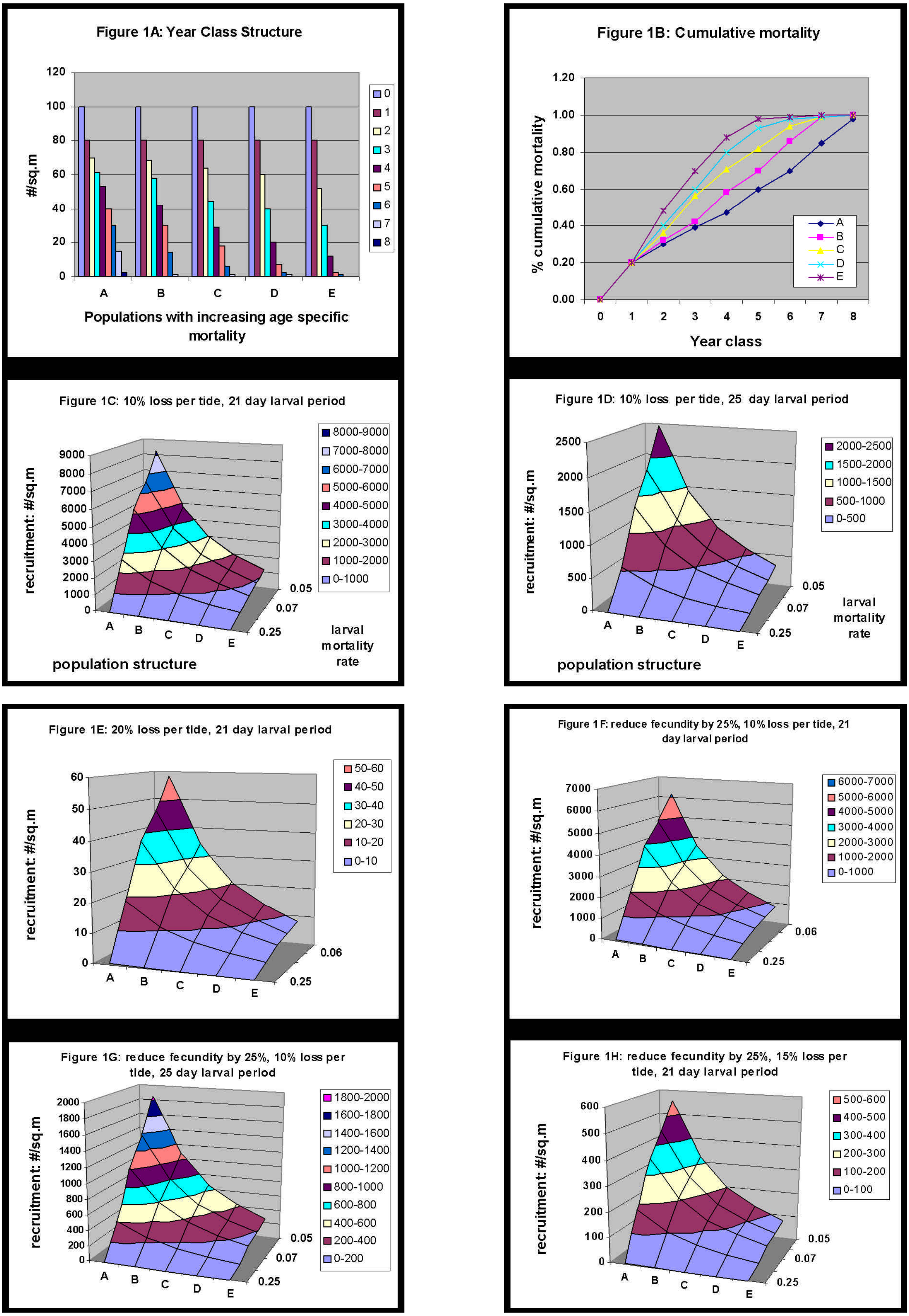

Fig. 1. Virtual oyster population year class structure (1A) and cumulative mortality (1B). Estimated recruitment (1C-1H) is shown under a variety of tidal loss, larval duration, and reduced fecundity scenarios

response may be strongly compromised. For example, even moderate disease tolerance alone will not be effective under sustained disease pressure, and restoration based only on this improved trait will fail. In practical terms, failure to effect restoration in the optimal location will result in failure in recruitment. Optimal location is a product of species traits that are arguably very conservative because of the evolution of the species (again see McEdwards 1995, Hall and Wake 1999) in combination with circulation patterns of the host estuary-a unique feature. Critical oyster traits in this mix include, but are not limited to, adult egg production, a trait for which we have not actively selected in breeding programs to date, and larval feeding ability and swimming behavior in turbid conditions. Fecundity is critical to driving the simulation as shown, yet we know essentially nothing of size-fecundity relationships under challenging conditions in which we are attempting restoration. Both larval traits are arguably very highly conserved because of limitations in the velar structure and the clear selective pressure over time for larval forms that recruit in optimal rather than sub-optimal environments. Turbid conditions can be viewed as transitions in the ephemeral lives of estuaries on a geological time frame, signals for oyster populations to move as they have done over periods of sea level rise. Larvae have no reason to evolve to survive in regions doomed to local extinction by rapidly changing environments. Their conserved feeding abilities and behavioral strategies have served them more than adequately without such abilities. Restoration efforts thus match a suite of larval traits with conditions that we strongly suspect are very far from optimal, yet we often proceed in the absence of knowledge as to how debilitating this mismatch may be to the desired end point. These troubling scenarios, well founded in both our current understanding of the evolution of complex life history and simple numerical simulations of recruitment processes in virtual populations under near optimized conditions, drive the need for focused research to address these deficiencies. Without quantitative descriptors of the response of each life history stage to the local stresses, their subsequent holistic synthesis in a practical model, and employment of sensitivity analyses, the options for adaptive management of long term, often very costly restoration efforts, are limited, indeed sobering and probably doomed to failure. Intensive aquaculture, by comparison, may be able to progress in marginal environments where delicate early life history stages are cultured in controlled situations, thus limiting mortality, before transfer to open systems. Fishery enhancement resides between these options, where a dual role of supplementing local reproduction is balanced against increased exploitation of commercial product.

Acknowledgements

This manuscript represent_s the result of cumulative effort over many years from several funding sources including National Oceanic and Atmospheric Administration (NOAA) Oyster Disease Program Grant number NA26FL0385-01, Chesapeake Bay Stock Assessment Program grant number NA07FU0539, Virginia Sea Grant number 115845-GL_I0003, National Science Foundation grant number OCE-9810624, and EPA Chesapeake Bay Program award number CB-983649-01-0. Preparation of the manuscript was supported by the Reves Center for International Studies at the College of William and Mary. Partial travel support to attend the 31^{st} United States-Japan Cooperative Program in Natural Resources (UJNR) Aquaculture Panel Meeting was provided by NOAA. These sources of funds are gratefully acknowledged. This manuscript is dedicated to my son, Matthew Francis Mann, who also attended the UJNR meeting, for his continuing intellectual curiosity and reminding me that science should, above all things, be fun. This is Contribution Number 2590 from the Virginia Institute of Marine Science.

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水研センター研報，別冊第１号，143－151，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 143-151, 2004

Integrated aquaculture systems for nutrient reduction in agricultural wastewater: potential and challenges*

Andrew M. LAZUR*[1] and Frank LETEUX*[2]

Abstract The integration of aquaculture with agriculture, such and fish production with poultry , has been practiced for hundreds of years and takes advantage of the nutrient output of one crop to increase pond primary productivity, subsequently, enhancing herbivorous fish production. Applying this integration concept for the purpose of reducing the environmental impact of agriculture via the nutrient extraction ability of various shellfish, plant and fish species, is however, a relatively new concept and is increasingly justified by nutrient discharge regulations and associated increasing effluent treatment costs. Agricultural operations, such as animal feedlots, are specific nutrient point sources in which integration with extraction aquaculture could reduce environmental impact. In addition to playing a key role in nutrient reduction, extraction aquaculture species can be an important source of income, critical to offsetting increasing nutrient treatment costs and increasing farm profitability. This paper will review current strategies to apply this concept in the field, present an overview of specific efforts in Florida and summarize the challenges of implementation of integrating production of various aquaculture species to reduce nutrients in agricultural wastewater.

Key words: Integration, aquaculture, agriculture, nutrient reduction

Agriculture has increasingly been identified as a major source of nitrogen and phosphorus contributing to eutrophication of numerous bodies of water worldwide. Traditional grain and vegetable row crop production with nitrogen and phosphorus based fertilizer applications, accounts for the majority of non-point nutrient sources in many watersheds including the Chesapeake Bay and Florida Everglades, USA (Boesch *et al.*, 2001; Sharpley, 2002; SFWMD, 2002a). The vastness of land dedicated to this type of agriculture presents a challenge for specific nutrient discharge treatment. However, progress in reducing nutrient runoff has been achieved with adoption of specific changes in production practices, or best management practices, such as no till seeding or planting winter cover crops to minimize soil erosion and enhance plant absorption of nutrients (Staver and Brinsfield, 1998; SFWMD, 2001a). Concentrated animal feeding operations (CAFO's) such as dairies, poultry houses, cattle and swine feedlots, and aquaculture, however, are finite point sources of nutrients, and therefore, specific nutrient capture or treatment systems could be more easily employed.

Numerous examples of aquaculture integrated with agricultural crop and animal production exist including fish culture with rice, fruit, ducks, chicken production and have been for hundreds of years in Asia (Beveridge and Little, 2002; Little and Edwards, 1998; Little

2003年６月12日受理（Received on June 12, 2003)
*[1] University of Maryland Center for Environmental Sciences, Horn Point Laboratory, P.O. Box 775, Cambridge, MD 21613 USA
*[2] Florida Department of Agriculture and Consumer Services, Office of Agricultural Water Policy, 1203 Governor's Square Blvd., Suite 200 Tallahassee, FL 32301 USA
* Presented in the Satellite Symposiun held in Shiogama on Oct. 21, 2002.

and Satapornvanit, 1996). In these cases, the goal is to maximize food production by utilizing waste from one crop to enhance production of another, in effect increasing nutrient utilization, and consequently, and until recently unintentionally, reducing nutrient impacts. Aquaculture species such as clams, mussels, oysters, algae and macrophytic plants are relatively efficient in assimilating or extracting nitrogen and phosphorus from their environment. (Boyd, 1995; Jones et al., 2002; Lasalle and Posadas, 1997; Newell, 1988; Schwartz and Boyd, 1995). In addition, numerous studies have shown the benefits of applying polyculture or culture of numerous species to reduce the nutrient and suspended solid loading of aquaculture effluent (Drenner, *et al.*; Hopkins *et al.*, 1993; Jones *et al.*, 2002; Schwartz and Boyd, 1998). The field of aquaponics, integrating fish and plant production, whereby fish wastes are used as fertilizer for vegetable crops has demonstrated successful method of increasing nutrient utilization and improving farm income (Racocy, *et al.*, 1992; and Diver, 2000). Using this extraction attribute of aquatic species in conjunction with agricultural crops specifically to reduce nitrogen and phosphorus discharges, or aquaculture serving as environmental rededication, is still in the development phase with limited field applications and research.

Current nutrient loading and reduction strategies in Florida

In south Florida agriculture runoff has been identified as the major contributor of the eutrophication of the Everglades and its watershed including Lake Okeechobee, Florida's largest lake. Phosphorus is the key limiting nutrient in freshwater systems and increases in phosphorus loading play a major role to eutrophication of the Everglades. SFWMD (2002a) estimated the total phosphorus import to the Lake Okeechobee watershed is over 1,800 tons per year originating from the major uses of the watershed including: improved pasture (33 %); truck crops (32 %); dairy (27 %); and citrus (11 %).

Recent nutrient management strategies focusing on phosphorus reductions include establishment of total maximum daily loads (TMDL's) for industries and CAFOs including an effluent standard of 40 ppb phosphorus for farm discharges. The use of best management practices (BMP's) and the employment of best available technologies (BATs) for many of the agricultural commodities including, beef cattle, poultry, citrus, and dairy are being established to achieve the new discharge standard (SFWMD, 2002b). Attempts to reduce phosphorus loading on a large watershed scale in the Lake Okeechobee region include: establishment of artificial wetlands, large chemical treatment ponds (which pump and inject untreated water with limestone, iron, humic acid, alum or polymers to bind P prior to discharging water), soil treatments, limiting nutrient applications, confinement barn-based technology, on-farm storage or retention (permanent storage) ponds, and governmental buy out of dairies (SFWMD, 2001b). These methods are very often costly to construct and maintain, have specific site limitations, and in the case of chemical treatment ponds and wetlands, have only temporary benefits due to substrate saturation and siltation. Dairies, which often concentrate several hundred to nearly two thousand animals per barn or dairy facility, have been identified as major phosphorus importers to Lake Okeechobee.

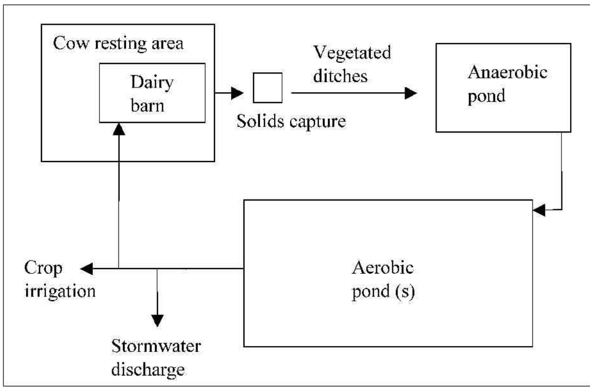

Fig. 1. Diagram of wastestream treatment and partial water re-use of a typical south Florida dairy

Using daily nutrient excretion estimates of Van Horn, *et al.* (1998) of 0.27-0.46 kg N and 0.05-0.068 kg P per dairy cow, total daily nutrient loading of barns in the Lake Okeechobee watershed are 81-828 kg and 15-122 kg of N and P respectively. They further show that solids collection, composting and crop irrigation of pond treated manure can capture significant levels of N and P and crops of bermudagrass hay and alfalfa periodically irrigated with dairy barn effluent resulted in 20-34 % and 22-49 % absorption of N and P respectively for the two crops.

Since the 1980's, the predominant method for treating CAFO wastes and effluent has been the use of individual or a series of lagoons or detention (temporary storage) ponds, both anaerobic and aerobic. The degree of nutrient reduction from ponds is highly variable and depends on many factors such as pre-pond solids capture, pond depth, surface area, oxygen concentrations, littoral area, plant species and hydraulic retention time. Increasing concern and government regulation of dairy effluent has prompted the necessity for additional or alternative nutrient reduction practices. Many dairies have increased the scale of treatment ponds and partially recycle water from the aerobic ponds for use as barn wash down water and for crop irrigation (Fig. 1). A waste treatment practice similar to the one depicted in Fig. 1 has resulted in reductions of 50 % and 76 % for N and P respectively with the majority of the remaining nitrogen being volatilized (Van Horn , *et al.*, 1998). The present challenge for this treatment practice is dealing with storm water discharge which is of special concern in the area due to low elevation and periodic hurricanes. New discharge requirements with a total maximum daily discharge (TDML) of 40 ppb phosphorus will mandate farms to treat storm induced off-site discharges as well, requiring additional treatment innovation and investment.

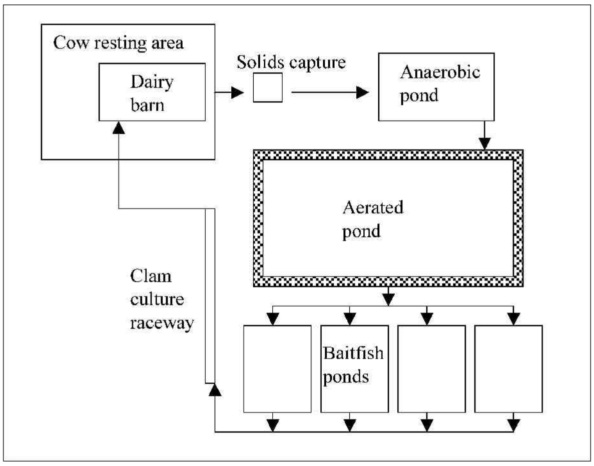

Fig. 2. Conceptual diagram of a integrated dairy - aquaculture operation with multi-stage water treatment and crop production. Shaded are in the aerobic pond depicts aquatic plant littoral zone.

The integrated dairy/aquaculture approach

One approach being developed for dairies in the Lake Okeechobee area involves incorporating culture of aquatic species such as Tilapia, paddlefish, baitfish (fish used as bait for recreational fishing), freshwater clams, and plants to provide additional nutrient assimilation, and in addition produce secondary crop income (Fig. 2). In this scenario, modifications to the water treatment ponds and ditches would be necessary as follows: 1) addition of more efficient solids removal equipment; 2) reshape pond bottom and slopes of anaerobic pond to facilitate removal of sludge accumulation in anaerobic pond; 3) renovate aerobic pond bottom to allow periodic fish harvesting; and 4) modify ditches and to enable culture and periodic harvest of the freshwater clam Corbicula fluminea.

With the modifications in place several phases of water treatment and nutrient reduction would occur:

Stage 1-Solids Collection:

Solids collection via use of a screen separator can remove 20-30 % of the organic matter from manure flushed with water that averages 72 % moisture and is comprised of 1-1.6 % N and 0.12-0.15 % P on a dry matter basis (Van Horn *et al.*, 1998). They also report an average of 45 kg of feces is defecated per cow daily. Taking into account the screen separator collection rate and the moisture and phosphorus content of organic matter, a reduction of approximately 25 % of the phosphorus or 0.004 kg P/cow is possible.

Stage 2-Anaerobic Pond:

Approximately 60 % of the nitrogen can be volatilized from an anaerobic pond via denitrification depending on hydraulic retention time. Phosphorus, however, is not removed by any organic mechanism within the anaerobic pond, but will accumulate into the bottom sludge. Phosphorus reduction estimated up to 50 % (Van Horn *et al.*, 1998) can occur when sludge is periodically collected and removed from the pond.

Stage 3-Aerobic Ponds:

Phosphorus absorption or removal can occur by several mechanisms within the aerated pond: fish harvest, macrophytic plant absorption, absorption by the pond sediment and phytoplankton. The aerobic ponds should receive intense mechanical aeration to enhance oxidation processes and to ensure adequate oxygen levels for the Tilapia stocked into the pond. The Tilapia serve as a forager on plankton and detritus, hence removing both nitrogen and phosphorus from the water. Harvesting Tialpia 2-3 times per year is possible and can be processed into a fish meal source for animal feeds. With a phosphorus and nitrogen content of Tilapia of 1.6 % and 2.7 % live weight respectively (Lovell, 1989) and using mean annual production of Tilapia of 1,600 kg/hectare/yr in cow manure fed ponds (Racocy and McGinty, 1989), an estimated 25.5 kg P and 43 kg N ha^{-1} y^{-1} removal is achieved.

In addition, the aerated pond has a littoral zone where aquatic plants can assimilate or extract additional phosphorus. Total export of P will depend on plant species and plant area. Common native emergent plant species which are effective in assimilating nutrients and have value in mitigation markets include: duck potato and arrowhead, *Sagittaria* spp., pickerelweed, *Pontederia* spp., and many shoreline grasses or rushes such as bulrush, Scirpus spp., and *Juncus* spp. Mitsch and Gosselink (2000) presented potential nutrient retention rates per area of marsh or wetland ranging from 3 -66 g N m^{-2} y^{-1} and 0.36-5.6 g P m^{-2} y^{-1}. In existing dairy operations, most of the aerobic ponds range from 4 -14 hectares in size and utilize 4 : 1 pond levee slopes, thus providing approximately 450 square meters of littoral zone per hectare of pond. Using nutrient retention rates suggested by Mitsch and Gooselink, nutrient retention would amount to 0.5-5.6 kg P ha^{-1} y^{-1} and 3-65 kg N ha^{-1} y^{-1}. Aquatic plants can also serve as an important secondary crop for sale in the restoration or mitigation markets.

Phosphorus readily binds with soil particles and accumulates in aerobic pond bottom muds. Pond sediment becomes saturated with phosphorus, which can be released into the water when the bottom sediments become anaerobic (Newell, 2003). The long-term P removal by the pond sediment will vary on several factors including degree of oxygenation, pH, soil type and plant absorption, but would be insignificant compared to other sources of P removal mechanisms in the aerobic pond.

Phytoplankton are efficient utilizers of phosphorus. Nurdogan and Oswald (1995) report 10-30 % absorption of phosphorus by microalgae in high-rate holding ponds and studies by UNEP (1999) show a 30-50 % uptake of P by microalgae in the aerobic pond portion of a waste stabilization pond system. The phosphorus uptake of microalgae is significant and appreciably greater than the potential from fish harvesting and marcrophytic plants in the perimeter littoral zone.

Stage 4-Baitfish Ponds:

Within the baitfish production ponds, species such as golden shiner, *Notemigonus crysoleucas* and bull minnow, *Fundulus* spp. feed on phytoplankton and zooplankton, and with harvesting further N and P assimilation and export from the system is achieved. Expected annual production of golden shiners using the wild spawn method (low fish yield culture practice where broodfish and offspring are raised in same pond) and with no commercial feed added is 110-330 kg ha^{-1} (Stone *et al.*, 1997).

Phosphorus removal rates from harvesting baitifsh would be expected to range from 1.8-5.3 kg ha^{-1} y^{-1}. In addition, the baitfish ponds proposed to be 4 hectares in size each would have similar littoral zone area and nutrient absorption as the aerobic pond on a per hectare basis. As in the aerated pond stage, phtoplankton would be present in the baitfish ponds and, therefore would be a major means of P uptake. In addition, incorporating the minor stocking (1-20 fish/ha) of paddlefish, *Polydon spathula*, an efficient zooplankton consumer, contributes to nutrient reduction benefit and also offers high value for its meat and roe. Paddlefish cultured in a 5 hectare reservior within a 200 hectare cattle watershed have been shown to survive for over 15 years and reach up to 36 kg (Kahrs, J., personal communication, 2002).

Stage 5-Clam Culture:

The final phase of water treatment prior to returning to the barn for wash down purposes, is clam culture within modified ditches or earthen raceways to facilitate periodic clam harvest. The species considered to be a good candidate for the integrated system in Florida is the Asian clam, *Corbicula fluminea*, due to its abundance throughout Florida freshwater bodies, tolerance to various water quality, high standing densities of up to 4,000 adults m^{-2}, relative high shell growth rate and rapid population recovery (McMahon, 1999). In addition, this species of clam has been observed to be a major feeder of phytoplankton, reducing plankton concentrations by 20-75 % in certain areas of the Potomac River (Cohen *et al.*, 1984). Unlike the fish culture portion of this integrated dairy/aquaculture system, in which extensive production data exists, the effectiveness and applicability of *Corbicula* as the final phase of water treatment within this integrated setting remains unproven. Current studies at the University of Florida Department of Fisheries and Aquatic Sciences are investigating some fundamental questions on *Corbicula* prior to incorporation into the dairy/aquaculture system. These include: substrate suitability, growth and filtering rates in a raceway environment, phosphorous uptake, shell and tissue P concentrations, and recommended water flow and clam stocking rates. Preliminary results show reasonable clam growth, but also a partial die off occurred which may be due to high water temperatures as observed with *Corbicula* in many natural environments (McMahon, 1999).

Summary of opportunities and challenges of integrated systems

The nutrient assimilation or reduction capability and production potential of all components within the integrated concept except the *Corbicula* stage have individually been demonstrated in the field. Using the mean P uptake values reported for stages 1 - 4 : 25 %, 50 % 20 %, and 40 % respectively, a 99 % total uptake may be possible without including the less significant removal by the Tilapia and baitfish harvesting as well as the littoral zone contributions. The *Corbicula* culture component would serve as the final P removal or polishing stage providing additional P removal. Proper sizing of each stage to the total P input or loading, would be necessary to prevent phosphorus being removed at high enough rates to starve subsequent stages of either P or P induced phytoplankton. Reviewing the uptake efficiencies, one could suggest that perhaps improvements on stages 1 , 2 and the contribution of phytoplankton together can accomplish P reduction goals. However, the fish, macrophytic plants and clams serve two important roles: 1) consumption of phytoplankton and subsequent removal of the P bound in the plankton via periodic harvesting; and 2) provide economic value to potentially pay for the treatment system. Pilot field studies are a essential tool to validate nutrient removal contributions of each stage and provide critical information in sizing the system, identifying optimum combinations of stages, and cost-benefit data.

The fundamental concept of sustainable animal production integration is maturing and being considered in many areas to improve sustainability of intensive animal production. Fedler and Parker (1994) present a similar multistage wastewater treatment scenario for swine or cattle production. In their system concept, fish, algae, duckweed, Lemma spp., and macrophytic plants are used to filter nutrients and, energy recovery, in the form of methane is incorporated. Parker and Fedler (1996) also note the increased potential for energy production through use of advanced facultative pond incorporated into an integrated wastewater treatment system and further suggest the many environmental and economic benefits of an integrated feedlot system.

The integrated concept is relatively simple in design, is applicable to a variety of type and scale of animal operations, and offers farmers the potential for increased farm income through sales of high value aquaculture crops. Incorporating a phosphorus reduction approach within the existing dairy barn treatment operations in south Florida offers cost savings and is more attractive to farmers compared to either new treatment systems or "regulatory relief" technologies, such as chemical treatment, that offers P reduction without any secondary crop income potential. Utilizing an aquaculture production component, does however, present challenges, especially the need for aquaculture training and experience, and detailed market information. In most cases, as was indicated by dairy farmers in the Lake Okeechobee area, demands on the farmers' time is currently too great to take on the additional time for training or production management responsibilities. Therefore, integrated operations would need to hire experienced individuals or contract with an aquaculture service company to manage the aquaculture component. An additional, and essential consideration is identification of market opportunities for the aquaculture products. The relatively large volume of fish and aquatic plants potentially generated by this system would be best accommodated through wholesale type markets rather than retail which require greater time due to smaller volumes and distances between customers. Thorough research on market outlets and their needs such as species preferences, product sizes, frequency of supply, volumes, seasonality of demand and pricing information is critical to evaluating the economic benefit of the aquaculture component and in the decision making process of investment.

The advantage of application in areas like south Florida are: 1) a long growing season of 10−11 months enabling efficient filtration capability of aquatic species nearly year-round; 2) the close proximity to high value markets and established distribution for baitfish (Adams *et al.*, 1998; Lazur, 1995), and aquatic plants; and 3) access to infrastructure and outlets using farm biosoilids such as, manure for compost, organic fertilizer, or bio energy production, and Tilapia and possibly clam shells for generation of animal feed products. Adapting this technology to cooler climates would result in reduced filtration efficiencies of aquatic species due to dormancy periods, which would increase the need for manure storage during winter months. However, the nutrient reduction benefit of wastewater treatment of an integrated system, despite the reduced growing season, may be highly significant and be more environmentally and economically justifiable than other options.

The culture of brackish water or marine aquatic algal and shellfish species with a joint goal of food production and nutrient reduction is expanding rapidly and Costa-Pierce, (2002) states many estuary based examples worldwide. Neori *et al.* (2000) studied a land-based marine recirculating polyculture system incorporating gilthead sea bream, *Sparus aurata*, Japanese abalone, *Halotis discus hannai*, and two seaweed species (as biofilters), *Ulva lactuca* and *Gracilaria conferta*. In their system, nutrients from the fish wastes were fed to the seaweed which was then fed to the abalone. They found that *Gracilaria* performance was poor, but the sea bream, *Ulva*, and abalone experienced good growth and the system converted 23 % of the nitrogen into fish and abalone flesh and an additional 10.3 % into seaweed exported from system. Based on their study, a component ratio of 0.6: 1 : 2 m^2 of abalone: fish: seaweed respectively is recommended. With this ratio, they estimate that 27% of the nitrogen would be converted to fish and abalone flesh with much of the remaining nitrogen in the form of ammonia and DON and would be recycled into the seaweed biofilters.

Applying marine species in integrated agriculture/aquaculture presents greater challenges given that CAFO's utilize freshwater and many operations are located inland. Those operations located near marine environments would require mixing the freshwater effluent with the marine water source in order to provide salinities required for the desired marine organisms. Recycling the treated water containing high salinities may not be acceptable in some applications, but may fit the dairy model and wash down use described within this paper.

The methods and potential combinations of components of integrating agricultural systems for nutrient reduction are numerous. The one concept presented here bases the nutrient reduction potential on a collection of observations and data for individual systems components. Field tests or demonstration projects are essential next steps and would provide valuable data in determining individual component and whole system nutrient reduction efficiencies, component compatibility, recommended nutrient input to component sizing, system costs and economic viability.

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水研センター研報，別冊第１号，153－154，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 153-154, 2004

Understanding the influence of bivalve suspension-feeder populations on water quality in eutrophic coastal waters

Roger I.E. NEWELL*, Jeff C. CORNWELL*, Raleigh R. HOOD* and Evamaria KOCH*

Suspension feeding bivalve molluscs serve to couple pelagic and benthic processes because they filter particles with high efficiency from the water column and transfer undigested remains in their biodeposits to the sediment surface. This feeding activity, combined with their often high abundance as natural stocks and in intensive aquaculture farms, can make bivalves extremely important in regulating water column processes in shallow coastal waters. Of all bivalve species worldwide, eastern oysters are among the most powerful in this regard because of their unusually high weight specific filtration rates (7 to 10 L h^{-1} g^{-1} dry tissue weight at typical summer water temperatures of 25 ℃.) The eastern oyster is well adapted to living in estuaries where inorganic particles comprise a large fraction of the seston because it can sort filtered particles prior to ingestion and reject less nutritious particles as pseudofeces. Currently in the nutrient enriched Chesapeake Bay, where phytoplankton are in high abundance, eastern oysters maintain high filtration rates but now reject large amounts of undigested algal cells in their pseudofeces. Newell (1988) initially drew attention to the possible ecosystem benefits of the original huge stocks of eastern oysters in Chesapeake Bay by comparing water column turnover times before oysters were commercially exploited to the situation today when oysters are at an all time low abundance.

In contrast to Newell's (1988) proposition that oyster populations may once have exerted "top-down" control on phytoplankton stocks others claim that oysters may simply recycle inorganic nutrients rapidly back to the water column and hence there would not have been any long-lasting reduction in phytoplankton biomass. To help distinguish between these scenarios, Newell *et al.* (2002) explored in laboratory incubations changes in nitrogen fluxes and denitrification under anoxic and oxic conditions in response to loading by different amounts of phytoplankton cells, representing an experimental analog of oyster biodeposits. When organics were regenerated under aerobic conditions, typical of those associated with shallow water oyster habitat, coupled nitrification-denitrification was promoted, resulting in denitrification of ～20 % of the total added nitrogen. In contrast under anoxic conditions, typical of current summertime conditions in main-stem Chesapeake Bay where phytoplankton is microbially degraded beneath the pycnocline, nitrogen was released solely as ammonium from the added organics. Such denitrification of particulate nitrogen remaining in the biodeposits of benthic suspension feeders will enhance nitrogen removal from eutrophied coastal waters (Newell, 2004).

It is likely that reduced oyster filtration by the much diminished oyster populations has contributed, in part, to observed higher turbidites in Chesapeake Bay and the consequent reduction in light reaching the sediment surface. In aerobic incubations of sediment cores with even low light levels (70 μmol^{-2} s^{-1}), Newell *et al.* (2002) found that a benthic microalgal/cyanobacterial community grew that not only absorbed the inorganic nitrogen released from oyster biodeposits but also fixed N2. This suggests that an

* Horn Point Laboratory, University of Maryland Center for Environmental Science, PO Box 775, Cambridge, MD 21631 USA
Email: newell@hpl.umces.edu

ecosystem dominated by benthic primary production may develop in shallow waters when reduced turbidity associated with bivalve feeding increases light penetration to a level that can sustain benthic microalgal production.

Over the last four decades seagrass beds have either declined or have disappeared throughout much of the Chesapeake Bay due to high water turbidity leading to reduced light availability for these benthic plants. In order to explore the possible interactions between oyster and seagrass declines we have developed a numerical model to simulate the interaction between wave-induced sediment resuspension, bivalve filtration, and seagrass growth. This model, which is parameterized based upon direct measurements of oyster filtration and seagrass wave dampening effects, shows that under high wave height conditions the presence of oysters can reduce suspended sediment concentrations by nearly an order of magnitude, which significantly increases water clarity and the depth to which seagrasses can grow (Newell, 2004).

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水研センター研報，別冊第１号，155，平成16年
Bull. Fish. Res. Agen. Supplement No. 1, 155, 2004

DEVELOPMENT OF A NOVEL GENE TRANSFER METHOD IN *PORPHYRA*

Chum-Mean LIN*[1], Charles YARISH*[2] and Thomas CHEN*[1]

Foreign genes have been successfully transferred into both animal and plant by transgenesis, but introduction of foreign genes into many important algae species is still in its infancy. Although nuclear and organelle transformations in *Chlamydomonas reinhardtii*, a green microalgal species, have been well established, attempts to develop transformation methods for macroalgae, such as *Volvox carteri* and several diatom species have just begun. Currently, there is no report of success of genetic transformation in *Porphyra* species (seaweed).

To facilitate applications of seaweed in both basic research and integrated aquaculture biotechnology, we have undertaken initiatives to develop gene transfer technology for Porphyra, and made significant progress. A plasmid DNA with a selection marker was transferred into archaeospores of *P. leucosticta* by electroporation. Total DNA was then prepared from those electroporated samples at various times of incubation including 24 hrs, one week, two weeks and up to two years. PCR analysis showed the consistent presence of plasmid DNA in all samples tested, strongly suggesting the success of gene transfer in Porphyra. The thalli developed from those transformed archaeospores grow well under continuous presence of selection pressure over two years with serial transfers. Reverse transcription (RT)/PCR analysis of RNA samples isolated from transgenic thalli showed expression of the transgene. These observations provide us with great confidence that genetic manipulation and production of transgenic Porphyra is feasible.

*[1] Department of Molecular and Cell Biology, and *[2] Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269 USA *Corresponding author, Email: tchen@uconnvm.uconn.edu